PHARMACEUTICAL COMPOSITIONS COMPRISING OX40 TARGETING ANTIBODIES AND USES THEREOF

1. Reference to Sequence Listing Submitted Electronically

[0001] This application incorporates by reference a Sequence Listing as an XML file entitled "938A007WO01P_SL" created on March 1, 2024 and having a size of 32,641 bytes.

2. Field

[0002] The present invention relates to molecular biology and pharmaceutical sciences. Provided herein are pharmaceutical compositions, formulations and dosage forms containing high concentrations of a monoclonal OX40 antibody and uses thereof in treating OX40-associated diseases.

3. Background

[0003] OX40 (also known as CD134) is a co-stimulatory molecule expressed on the surface of activated T cells. It plays a crucial role in regulating the immune response, particularly in the activation and proliferation of T cells. Antibodies against human OX40 receptor (OX40) have been developed for treating various diseases, such as autoimmune diseases, inflammatory diseases, or other disorders, such as cancer.

[0004] Stable pharmaceutical compositions having the anti-OX40 antibody at high concentrations which can be suitable for, *e.g.*, subcutaneous injection, are in particular need. Due to the varying properties of active drug substances being formulated and potential interaction between different excipients, dosage forms typically require pharmaceutical excipients that are uniquely tailored to the active drug substance in order to achieve advantageous physical and pharmaceutical properties. The compositions provided herein are so formulated that pharmaceutical excipients contained therein serve tailored and specialized pharmaceutical functions, *e.g.*, solubilization, dilution, thickening or thinning, stabilization, preservation, *etc.*

4. Summary

[0005] Provided herein are pharmaceutical compositions comprising (A) an anti-OX40 antibody or antigen-binding fragment thereof comprising (1) a light chain variable region (VL) comprising VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:1, 2, and 3, respectively; and (2) a heavy chain variable region (VH) comprising VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of SEQ ID NOs:4, 5 and 6, respectively; and (B) a pharmaceutically acceptable carrier comprising a glutamic/histidine buffer, proline, and polysorbate

80. In some embodiments, (1) the VL has at least 85%, at least 90%, at least 95%, at least 98%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:7; and/or (2) the VH has at least 85%, at least 90%, at least 95%, at least 98%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:8. In some embodiments, the VL has the amino acid sequence of SEQ ID NO:7, 9, or 10, and the VH has the amino acid sequence of SEQ ID NO:8, 11, or 12. In some embodiments, the VL and VH have the amino acid sequences of SEQ ID NOs:7 and 8, respectively.

[0006] In some embodiments, the anti-OX40 antibody or antigen-binding fragment is a chimeric antibody or antigen-binding fragment, a humanized antibody or antigen-binding fragment, or a human antibody or antigen-binding fragment. In some embodiments, the anti-OX40 antibody or antigen-binding fragment is a humanized antibody or antigen-binding fragment.

[0007] In some embodiments of the pharmaceutical compositions disclosed herein, the anti-OX40 antibody or antigen-binding fragment is selected from the group consisting of a Fab, a Fab', a F(ab')2, a Fv, a scFv, and a (scFv)2. In some embodiments, the anti-OX40 antibody or antigen-binding fragment is an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody.

[0008] In some embodiments of the pharmaceutical compositions disclosed herein, the anti-OX40 antibody is a humanized IgG1 antibody. In some embodiments, the anti-OX40 antibody comprises a light chain constant region (CL) having at least 85% sequence identity to kappa CL (Cκ; SEQ ID NO:13) or lambda CL (Cλ; SEQ ID NO:14). In some embodiments, the CL is Cκ (SEQ ID NO:13). In some embodiments, the anti-OX40 antibody comprises the wildtype heavy chain constant region (CH) of human IgG1 (SEQ ID NO:16), or a variant thereof having reduced binding to FcγR. In some embodiments, the anti-OX40 antibody comprises the CH region of human IgG1 with N297A substitution. In some embodiments, the anti-OX40 antibody comprises a light chain having the amino acid sequence of SEQ ID NO:19 and a heavy chain having the amino acid sequence of SEQ ID NO:20.

[0009] In some embodiments, pharmaceutical compositions provided herein comprise the anti-OX40 antibody or antigen-binding fragment at a concentration ranging from 80 to 180 mg/mL, from 100 to 180 mg/mL, from 120 to 180 mg/mL, from 140 to 180 mg/mL, from 100 to 160 mg/mL, or from 120 to 160 mg/mL. In some embodiments, pharmaceutical compositions provided herein comprise the antibody or antigen-binding fragment at a concentration ranging from 140 to 160 mg/mL. In some embodiments, pharmaceutical compositions provided herein comprise the antibody or antigen-binding fragment at a concentration of about 150 mg/mL.

[0010] In some embodiments, pharmaceutical compositions provided herein comprise the glutamic/histidine buffer at a concentration ranging from 10 to 50 mM, from 10 to 40 mM, from 10

to 30 mM, or from 15 to 25 mM. In some embodiments, pharmaceutical compositions provided herein comprise the glutamic/histidine buffer at 20 mM.

[0011] In some embodiments, pharmaceutical compositions provided herein comprise the proline at a concentration ranging from 1.0 to 5.0% (w/v), from 1.0 to 4.0% (w/v), from 1.0 to 3.0% (w/v), from 2.0 to 5.0% (w/v), from 2.0 to 4.0% (w/v), or from 2.0 to 3.0% (w/v). In some embodiments, pharmaceutical compositions provided herein comprise the proline at a concentration ranging from 2.5 to 3.0% (w/v). In some embodiments, pharmaceutical compositions provided herein comprise the proline at 2.8% (w/v).

[0012] In some embodiments, pharmaceutical compositions provided herein comprise the polysorbate 80 at a concentration ranging from 0.001 to 0.1% (w/v), from 0.005 to 0.05% (/v), or from 0.01 to 0.05% (v/v). In some embodiments, pharmaceutical compositions provided herein comprise the polysorbate 80 at a concentration ranging from 0.01 to 0.03% (w/v). In some embodiments, pharmaceutical compositions provided herein comprise the polysorbate 80 at 0.02% (w/v).

[0013] In some embodiments, pharmaceutical compositions provided herein have a pH ranging from 4.8 to 6.8, from 5.0 to 6.5, from 5.0 to 6.0, or from 5.0 to 5.8. In some embodiments, pharmaceutical compositions provided herein have a pH ranging from 5.1 to 5.6. In some embodiments, pharmaceutical compositions provided herein have a pH of about 5.3 or about 5.4.

[0014] In some embodiments, pharmaceutical compositions provided herein comprise 20 mM glutamic/histidine buffer, 2.8% (w/v) proline, and 0.01-0.03% (w/v) polysorbate 80, wherein the pH ranges from 5.0 to 5.6. In some embodiments, pharmaceutical compositions provided herein comprise 20 mM glutamic/histidine buffer, 2.8% (w/v) proline, and 0.02% (w/v) polysorbate 80, wherein the pH is about 5.3 or about 5.4.

[0015] In some embodiments, the pharmaceutical composition is in liquid form. In some embodiments, the pharmaceutical composition is lyophilized.

[0016] In some embodiments, the viscosity of the pharmaceutical composition is no more than 20 cP, no more than 18 cP, no more than 16 cP, no more than 14 cP, no more than 12 cP, or no more than 10 cP. In some embodiments, the viscosity is about 12 cP.

[0017] In some embodiments, the osmotic pressure of the pharmaceutical composition ranges from 200 to 400 mOsmol/kg, from 250 to 400 mOsmol/kg, or from 300 to 400 mOsmol/kg. In some embodiments, the osmotic pressure is about 350 mOsmol/kg.

[0018] In some embodiments of the pharmaceutical compositions provided herein, the purity of the antibody or antigen-binding fragment is at least 90%. In some embodiments, the purity of the

antibody or antigen-binding fragment is 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, or at least 99%. In some embodiments, the purity of the antibody or antigen-binding fragment is measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC) or Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS). In some embodiments, the purity of the antibody or antigen-binding fragment is at least 95% measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC). In some embodiments, the purity of the antibody or antigen-binding fragment is at least 90% measured by Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS).

[0019] In some embodiments, pharmaceutical compositions provided herein compare no more than 60% charge variants. In some embodiments, pharmaceutical compositions provided herein comprise no more than 55%, no more than 50%, no more than 45%, or no more than 40%, no more than 35%, or no more than 30% charge variants. In some embodiments, the charge variants are measured by Cation Exchange-High Performance Liquid Chromatography (CEX-HPLC).

[0020] In some embodiments, the purity of the antibody or antigen-binding fragment is reduced by no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%, or no more than 2% after storage for a period of time that is at least 2 weeks. In some embodiments, the purity is measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC) or Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS).

[0021] In some embodiments, the charge variants of the antibody or antigen-binding fragment are increased by no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5% after storage for a period of time that is at least 2 weeks. In some embodiments, the charge variants are measured by Cation Exchange-High Performance Liquid Chromatography (CEX-HPLC).

[0022] In some embodiments, the period of time is at least 2 weeks, at least 4 weeks, at least 2 months, at least 3 months, at least 6 months, at least 9 months, at least 12 months, at least 18 months, or at least 24 months. In some embodiments, the pharmaceutical compositions are stored at about -80°C, about -20°C, about 4°C, about 25°C or about 40°C. In some embodiments, the pharmaceutical compositions are stored upright or inverted. In some embodiments, the pharmaceutical compositions are stored with agitation.

[0023] In some embodiments of the pharmaceutical compositions provided herein, the purity of the antibody or antigen-binding fragment is reduced by no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%,

or no more than 2% after at least 1, at least 2, at least 3, at least 4, or at least 5 freeze-thaw cycles. In some embodiments, the purity is measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC) or Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS).

[0024] In some embodiments of the pharmaceutical compositions provided herein, the charge variants of the antibody or antigen-binding fragment are increased to no more than 60%, to no more than 55%, no more than 50%, no more than 45%, or no more than 40%, or no more than 35% after at least 1, at least 2, at least 3, at least 4, or at least 5 freeze-thaw cycles. In some embodiments, the charge variants are measured by Cation Exchange-High Performance Liquid Chromatography (CEX-HPLC).

[0025] In some embodiments, pharmaceutical compositions provided herein are suitable for subcutaneous injection.

[0026] In some embodiments, provided herein are methods of treating an OX40-associated disease comprising administering a therapeutically effective amount of the pharmaceutical compositions provided herein. In some embodiments, methods provided herein comprise subcutaneous injection.

[0027] In some embodiments, provided herein are uses of the pharmaceutical composition described herein in treating an OX40-associated disease. In some embodiments, provided herein are uses of the pharmaceutical composition described herein in preparation of a medication for treating an OX40-associated disease. In some embodiments, the OX40-associated disease is alopecia areata (AA) or atopic dermatitis (AD).

[0028] In some embodiments, provided herein are kits comprising the pharmaceutical compositions disclosed herein.

5. Detailed Description

[0029] Provided herein are stable compositions, formulations and dosage forms comprising monoclonal anti-OX40 antibodies at high concentrations. The compositions, formulations and dosage form provide several advantages, including, for example, suitability for subcutaneous administration.

[0030] The high order structures of the antibodies are very fragile and prone to structural changes, such as denaturation, aggregation, and precipitation. As such, preparation of antibody formulations can be challenging due to the need to maintain their structural integrity, activity, and stability over time. Some key challenges include protein aggregation, chemical degradation, and physical instability. Aggregation can lead to loss of activity and potential immunogenicity issues, which can occur due to various factors such as protein concentration, pH, temperature, and shear stress during

manufacturing processes. Chemical degradation, including oxidation, deamidation, and hydrolysis, can compromise the stability and efficacy of the antibodies. Factors such as pH, temperature, and presence of excipients can influence chemical degradation pathways. Physical degradation, including denaturation, unfolding, and conformational changes, can affect the binding affinity and biological activity of antibodies. Factors such as temperature fluctuations, freeze-thaw cycles, and agitation during handling and storage can contribute to physical instability. Lyophilization (freeze-drying) is commonly used to enhance the stability of antibody formulations. However, the process can introduce additional challenges, such as protein denaturation, aggregation, and loss of activity during freezing and drying cycles.

[0031] Selecting suitable excipients, including a buffering system to maintain the proper pH is critical for stabilizing antibody formulations. Despite the knowledge in the art, every antibody is unique in its structure and physicochemical properties and stability profile, which necessitates a tailored and creative approach to formulation development. For example, variations in amino acid sequence and post-translational modifications (PTMs), such as glycosylation, phosphorylation, and oxidation, can impact their stability and functional properties. The diverse biophysical properties, including isoelectric point (pI), hydrophobicity, and thermal stability, also influence their behavior in solution and susceptibility to aggregation and degradation. Formulation development involves characterizing these biophysical properties and designing formulation conditions that promote antibody stability and maintain the desired conformation and activity.

[0032] Additionally, excipients used in antibody formulations can interact with antibodies through electrostatic, hydrophobic, or steric interactions, leading to changes in protein stability, aggregation, or activity. The nature and extent of these interactions vary depending on the specific excipients and antibody characteristics, necessitating careful selection and optimization of excipient concentrations and composition to minimize adverse effects on antibody stability.

[0033] Subcutaneous administration offers several advantages over intravenous infusion, including convenience, reduced healthcare resource utilization, and improved patient compliance. However, high-concentration formulations are required to deliver therapeutic doses of the antibody efficiently via the subcutaneous route, which pose greater challenges due to various factors that can impact the stability, solubility, and aggregation propensity of the antibodies. For example, at higher concentrations, proteins have a higher probability of encountering and interacting with each other, and such protein-protein interactions can lead to the formation of aggregates through mechanisms such as reversible self-association or irreversible aggregation. Higher protein concentrations can also exacerbate stress-induced aggregation mechanisms, such as agitation, shear stress, and temperature

fluctuations. These stresses can promote protein unfolding, exposure of hydrophobic regions, and intermolecular interactions, leading to the formation of aggregates. Formulation strategies must be optimized to mitigate stress-induced aggregation and maintain protein stability under relevant manufacturing and storage conditions. Additional factors that are especially relevant for high concentration formulations include viscosity and osmotic pressure. Higher protein concentrations can result in increased solution viscosity, which can pose challenges during formulation, handling, and administration. High viscosity formulations can be difficult to filter, fill into vials or syringes, and administer to patients. Elevated viscosity can also affect the flow properties of formulations, potentially impacting drug delivery and distribution *in vivo*. Also, increasing protein concentration in a formulation can elevate the osmotic pressure of the solution, which can affect product stability and compatibility with packaging materials. Elevated osmotic pressure can lead to osmotic stress on protein molecules, potentially causing conformational changes, aggregation, or precipitation. Formulation optimization is necessary to balance osmotic pressure considerations with protein stability and product compatibility requirements.

[0034] Furthermore, higher protein concentrations can approach or exceed the solubility limit of the protein in the formulation buffer, leading to protein precipitation or crystallization. As such, additional formulation strategies, such as adjusting pH, adding stabilizing agents, or optimizing buffer conditions, can be required to maintain protein solubility at elevated concentrations.

[0035] Briefly, pharmaceutical compositions containing OX40 targeting antibodies at high concentrations hold significant promise, which also addresses the need for more convenient and effective therapeutic options while potentially improving patient outcomes and healthcare resource utilization. However, increasing protein concentration in a formulation presents significant formulation challenges, including protein-protein interactions, elevated viscosity, stress-induced aggregation, osmotic pressure effects, solubility limitations, etc. Addressing these challenges requires careful formulation optimization, excipient selection, and analytical characterization to ensure the stability, efficacy, and safety of high-concentration formulations of the antibody therapeutic. The pharmaceutical compositions provided herein address these challenges and provide related advantages, which can be used in, for example, treating, preventing and/or managing various disease or disorders, including, but not limited to, OX40-related diseases.

[0036] Before the present disclosure is further described, it is to be understood that the disclosure is not limited to the particular embodiments set forth herein, and it is also to be understood that the terminology used herein is for the purpose of describing particular embodiments, and is not intended to be limiting.

5.1 Definitions

[0037] Unless otherwise defined herein, scientific and technical terms used in the present disclosures 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. Generally, nomenclatures 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.

[0038] The term "a" or "an" entity refers to one or more of that entity; for example, "an antibody," is understood to represent one or more antibodies.

[0039] The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0040] As used herein, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects. The term "about" encompasses the exact number recited. In some embodiments, "about" means within plus or minus 10% of a given value or range. In certain embodiments, "about" means that the variation is ±5%, ±4%, ±3%, ±2%, ±1%, ±0.5%, ±0.2%, or ±0.1% of the value to which "about" refers. In some embodiments, "about" means that the variation is ±1%, ±0.5%, ±0.2%, or ±0.1% of the value to which "about" refers.

[0041] The term "antibody," and its grammatical equivalents as used herein refer to an immunoglobulin molecule that recognizes and specifically binds a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or a combination of any of the foregoing, through at least one antigen-binding site wherein the antigen-binding site is usually within the variable region of the immunoglobulin molecule. As used herein, the term encompasses intact polyclonal antibodies, intact monoclonal antibodies, single-domain antibodies (sdAbs; *e.g.*, camelid antibodies, alpaca antibodies), single-chain Fv (scFv) antibodies, heavy chain antibodies (HCAbs), light chain antibodies (LCAbs), multispecific antibodies, bispecific antibodies, monospecific antibodies, monovalent antibodies, and any other modified immunoglobulin molecule comprising an antigen-binding site (*e.g.*, dual variable domain immunoglobulin molecules) as long as the antibodies

exhibit the desired biological activity. Antibodies also include, but are not limited to, mouse antibodies, camel antibodies, chimeric antibodies, humanized antibodies, and human antibodies. An antibody can be any of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. Unless expressly indicated otherwise, the term "antibody" as used herein include "antigen-binding fragment" of intact antibodies. The term "antigen-binding fragment" as used herein refers to a portion or fragment of an intact antibody that is the antigenic determining variable region of an intact antibody. Examples of antigen-binding fragments include, but are not limited to, Fab, Fab', F(ab')2, Fv, linear antibodies, single chain antibody molecules (*e.g.*, scFv), heavy chain antibodies (HCAbs), light chain antibodies (LCAbs), disulfide-linked scFv (dsscFv), diabodies, tribodies, tetrabodies, minibodies, dual variable domain antibodies (DVD), single variable domain antibodies (sdAbs; *e.g.*, camelid antibodies, alpaca antibodies), and single variable domain of heavy chain antibodies (VHH), and bispecific or multispecific antibodies formed from antibody fragments. A "bispecific" antibody is an artificial hybrid antibody having two different antigen binding sites, which recognize and specifically bind two different targets. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. *See*, *e.g.*, Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-321 (1990); Kostelny *et al.*, *J. Immunol.* 148, 1547-1553 (1992).

[0042] The term "humanized antibody" as used herein refers to forms of non-human (*e.g.,* murine) antibodies that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human sequences. Typically, humanized antibodies are human immunoglobulin. In some instances, the Fv framework region residues of a human immunoglobulin are replaced with the corresponding residues in an antibody from a non-human species. In some instances, residues of the CDRs are replaced by residues from the CDRs of a non-human species (*e.g.,* mouse, rat, hamster, camel) that have the desired specificity, affinity, and/or binding capability. The humanized antibody can be further modified by the substitution of additional residues either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or binding capability. The term "human antibody" as used herein refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human made using any of the techniques known in the art.

[0043] The term "heavy chain" when used in reference to an antibody refers to a polypeptide chain of about 50-70 kDa, wherein the amino-terminal portion includes a variable region of about 120 to

130 or more amino acids and a carboxy-terminal portion that includes a constant region. The constant region can be one of five distinct types, referred to as alpha (α), delta (δ), epsilon (ε), gamma (γ) and mu (μ), based on the amino acid sequence of the heavy chain constant region. The distinct heavy chains differ in size: α, δ and γ contain approximately 450 amino acids, while μ and ε contain approximately 550 amino acids. When combined with a light chain, these distinct types of heavy chains give rise to five well known classes of antibodies, IgA, IgD, IgE, IgG and IgM, respectively, including four subclasses of IgG, namely IgGl, IgG2, IgG3 and IgG4. A heavy chain can be a human heavy chain.

[0044] The term "light chain" when used in reference to an antibody refers to a polypeptide chain of about 25 kDa, wherein the amino-terminal portion includes a variable region of about 100 to about 110 or more amino acids and a carboxy-terminal portion that includes a constant region. The approximate length of a light chain is 211 to 217 amino acids. There are two distinct types, referred to as kappa (κ) and lambda (λ) based on the amino acid sequence of the constant domains. Light chain amino acid sequences are well known in the art. A light chain can be a human light chain.

[0045] The term "variable domain" or "variable region" refers to a portion of the light or heavy chains of an antibody that is generally located at the amino-terminal of the light or heavy chains and has a length of about 120 to 130 amino acids in the heavy chain and about 100 to 110 amino acids in the light chain, and is used in the binding and specificity of each particular antibody for its particular antigen. The variable domain of the light chain is referred to as the "VL"; and the variable domain of the heavy chain is referred to as the "VH." The variable domains differ extensively in sequence between different antibodies. The variability in sequence is concentrated in the CDRs while the less variable portions in the variable domain are referred to as framework regions (FR). The CDRs of the light and heavy chains are primarily responsible for the interaction of the antibody with antigen. The numbering of amino acid positions used herein is according to the EU Index, as in Kabat *et al.* (1991) Sequences of proteins of immunological interest. (U.S. Department of Health and Human Services, Washington, D.C.) 5th ed. A variable region can be a human variable region.

[0046] A CDR refers to one of three hypervariable regions (H1, H2 or H3) within the non-framework region of the immunoglobulin (Ig or antibody) VH β-sheet framework, or one of three hypervariable regions (L1, L2 or L3) within the non- framework region of the antibody VL β-sheet framework. Accordingly, CDRs are variable region sequences interspersed within the framework region sequences. CDR regions are well known to those skilled in the art and have been defined by a variety of methods/systems. These systems and/or definitions have been developed and refined over years and include Kabat, Chothia, IMGT, AbM, and Contact. For example, Kabat defines the regions

of most hypervariability within the antibody variable (V) domains (Kabat *et al.*, *J. Biol. Chem.* 252:6609-6616 (1977); Kabat, *Adv. Prot. Chem.* 32:1-75 (1978)). The Chothia definition is based on the location of the structural loop regions, which defines CDR region sequences as those residues that are not part of the conserved β-sheet framework, and thus are able to adapt different conformations (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987)). Both terminologies are well recognized in the art. Additionally, the IMGT system is based on sequence variability and location within the structure of the variable regions. The AbM definition is a compromise between Kabat and Chothia. The Contact definition is based on analyses of the available antibody crystal structures. Software programs (*e.g.*, abYsis) are available and known to those of skill in the art for analysis of antibody sequence and determination of CDRs. The positions of CDRs within a canonical antibody variable domain have been determined by comparison of numerous structures (Al-Lazikani *et al.*, *J. Mol. Biol.* 273:927-948 (1997); Morea *et al.*, *Methods* 20:267-279 (2000)). Because the number of residues within a hypervariable region varies in different antibodies, additional residues relative to the canonical positions are conventionally numbered with a, b, c and so forth next to the residue number in the canonical variable domain numbering scheme (Al-Lazikani *et al.*, *supra* (1997)). Such nomenclature is similarly well known to those skilled in the art.

[0047] For example, CDRs defined according to either the Kabat (hypervariable) or Chothia (structural) designations, are set forth in the table below.

	Kabat[1]	Chothia[2]	Loop Location
VHCDRl	31-35	26-32	linking B and C strands
VHCDR2	50-65	53-55	linking C' and C'' strands
VHCDR3	95-102	96-101	linking F and G strands
VLCDRl	24-34	26-32	linking B and C strands
VLCDR2	50-56	50-52	linking C' and C'' strands
VLCDR3	89-97	91-96	linking F and G strands

[1]Residue numbering follows the nomenclature of Kabat *et al.*, *supra*

[2]Residue numbering follows the nomenclature of Chothia *et al.*, *supra*

[0048] One or more CDRs also can be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin. An immunoadhesin can incorporate the CDR(s) as part of a larger polypeptide chain, can covalently link the CDR(s) to another polypeptide chain, or can incorporate the CDR(s) noncovalently. The CDRs permit the immunoadhesin to bind to a particular

antigen of interest. The CDR regions can be analyzed by, for example, abysis website (http://abysis.org/).

[0049] The term "specifically binds," as used herein, means that a polypeptide or molecule interacts more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to the epitope, protein, or target molecule than with alternative substances, including related and unrelated proteins. A binding moiety (*e.g.*, antibody) that specifically binds a target molecule (*e.g.,* antigen) can be identified, for example, by immunoassays, ELISAs, Bio-Layer Interferometry ("BLI"), SPR (*e.g.*, Biacore), or other techniques known to those of skill in the art. Typically, a specific reaction will be at least twice background signal or noise and can be more than 10 times background. *See*, *e.g.*, Paul, ed., 1989, Fundamental Immunology Second Edition, Raven Press, New York at pages 332-336 for a discussion regarding antibody specificity. A binding moiety that specifically binds a target molecule can bind the target molecule at a higher affinity than its affinity for a different molecule. In some embodiments, a binding moiety that specifically binds a target molecule can bind the target molecule with an affinity that is at least 20 times greater, at least 30 times greater, at least 40 times greater, at least 50 times greater, at least 60 times greater, at least 70 times greater, at least 80 times greater, at least 90 times greater, or at least 100 times greater, than its affinity for a different molecule. In some embodiments, a binding moiety that specifically binds a particular target molecule binds a different molecule at such a low affinity that binding cannot be detected using an assay described herein or otherwise known in the art. In some embodiments, "specifically binds" means, for instance, that a binding moiety binds a molecule target with a K_D of about 0.1 mM or less. In some embodiments, "specifically binds" means that a polypeptide or molecule binds a target with a K_D of about 10 μM or less or about 1 μM or less. In some embodiments, "specifically binds" means that a polypeptide or molecule binds a target with a K_D of about 0.1 μM or less, about 0.01 μM or less, or about 1 nM or less. Because of the sequence identity between homologous proteins in different species, specific binding can include a polypeptide or molecule that recognizes a protein or target in more than one species. Likewise, because of homology within certain regions of polypeptide sequences of different proteins, specific binding can include a polypeptide or molecule that recognizes more than one protein or target. It is understood that, in some embodiments, a binding moiety (*e.g.,* antibody) that specifically binds a first target may or may not specifically bind a second target. As such, "specific binding" does not necessarily require (although it can include) exclusive binding, *i.e.,* binding to a single target. Thus, a binding moiety (*e.g.,* antibody) can, in some embodiments, specifically bind more than one target. For example, an antibody can, in certain instances, comprise two identical antigen-binding sites, each of which

specifically binds the same epitope on two or more proteins. In certain alternative embodiments, an antibody can be bispecific and comprise at least two antigen-binding sites with differing specificities.

[0050] The terms "polypeptide," "peptide," "protein," and their grammatical equivalents as used interchangeably herein refer to polymers of amino acids of any length, which can be linear or branched. It can include unnatural or modified amino acids or be interrupted by non-amino acids. A polypeptide, peptide, or protein can also be modified with, for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification.

[0051] The term "variant" as used herein in relation to a protein or a polypeptide with particular sequence features (the "reference protein" or "reference polypeptide") refers to a different protein or polypeptide having one or more (such as, for example, about 1 to about 30, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, or about 1 to about 5) amino acid substitutions, deletions, and/or additions as compared to the reference protein or reference polypeptide. The changes to an amino acid sequence can be amino acid substitutions. The changes to an amino acid sequence can be conservative amino acid substitutions. The changes to an amino acid sequence can be amino acid deletions. A variant can be a fragment of the reference protein or polypeptide. A functional variant of a protein or polypeptide maintains the basic structural and functional properties of the reference protein or polypeptide.

[0052] The terms "polynucleotide," "nucleic acid," and their grammatical equivalents as used interchangeably herein refer to a polymer or oligomer of nucleotides of any length. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases (such as methylated, hydroxymethylated, or glycosylated), non-natural nucleotides, non-nucleotide building blocks that exhibit similar structure and/or function as natural nucleotides (*i.e.*, "nucleotide analogs"), and/or any substrate that can be incorporated into a polymer by DNA or RNA polymerase. The nucleic acids or polynucleotides can be heterogenous or homogenous in composition, can be isolated from naturally occurring sources, or can be artificially or synthetically produced. In addition, the nucleic acids may be DNA or RNA, or a mixture thereof, and can exist permanently or transitionally in single-stranded or double-stranded form, including homoduplex, heteroduplex, and hybrid states. Nucleic acid structures also include, for instance, a DNA/RNA helix, peptide nucleic acid (PNA), morpholino nucleic acid (see, *e.g.*, Braasch and Corey, *Biochemistry*, 4(14): 4503-4510 (2002) and U.S. Patent 5,034,506), locked nucleic acid (LNA; see Wahlestedt *et al.*, *Proc. Natl. Acad. Sci.* U.S.A., 97: 5633-5638 (2000)), cyclohexenyl nucleic acid (see Wang, *Am. Chem. Soc.*, 122: 8595-8602 (2000)), and/or a ribozyme.

[0053] The terms "identical," percent "identity," and their grammatical equivalents as used herein in the context of two or more polynucleotides or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percentage identity can be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software that can be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include, but are not limited to, BLAST, ALIGN, Megalign, BestFit, GCG Wisconsin Package, and variants thereof. In some embodiments, two polynucleotides or polypeptides provided herein are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some embodiments at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some embodiments, identity exists over a region of the amino acid sequences that is at least about 10 residues, at least about 20 residues, at least about 40-60 residues, at least about 60-80 residues in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 residues, such as at least about 80-100 residues, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, such as the coding region of a target protein or an antibody. In some embodiments, identity exists over a region of the nucleotide sequences that is at least about 10 bases, at least about 20 bases, at least about 40-60 bases, at least about 60-80 bases in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 bases, such as at least about 80-100 bases or more, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, such as a nucleotide sequence encoding a protein of interest.

[0054] A polypeptide, peptide, protein, antibody, polynucleotide, vector, cell, or composition which is "isolated" is a polypeptide, peptide, protein, antibody, polynucleotide, vector, cell, or composition which is in a form not found in nature. Isolated polypeptides, peptides, proteins, antibodies, polynucleotides, vectors, cells, or compositions include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, a polypeptide, peptide, protein, antibody, polynucleotide, vector, cell, or composition which is isolated is substantially pure.

[0055] The term "treat" and its grammatical equivalents as used herein in connection with a disease or a condition, or a subject having a disease or a condition refer to an action that suppresses, eliminates, reduces, and/or ameliorates a symptom, the severity of the symptom, and/or the frequency of the symptom associated with the disease or disorder being treated.

[0056] The term "administer" and its grammatical equivalents as used herein refer to the act of delivering, or causing to be delivered, a therapeutic or a pharmaceutical composition to the body of a subject by a method described herein or otherwise known in the art. The therapeutic can be a compound, a polypeptide, an antibody, a cell, or a population of cells. Administering a therapeutic or a pharmaceutical composition includes prescribing a therapeutic or a pharmaceutical composition to be delivered into the body of a subject. Exemplary forms of administration include oral dosage forms, such as tablets, capsules, syrups, suspensions; injectable dosage forms, such as intravenous (IV), intramuscular (IM), or intraperitoneal (IP); transdermal dosage forms, including creams, jellies, powders, or patches; buccal dosage forms; inhalation powders, sprays, suspensions, and rectal suppositories.

[0057] The terms "effective amount," "therapeutically effective amount," and their grammatical equivalents as used herein refer to the administration of an agent to a subject, either alone or as a part of a pharmaceutical composition and either in a single dose or as part of a series of doses, in an amount that is capable of having any detectable, positive effect on any symptom, aspect, or characteristics of a disease, disorder or condition when administered to the subject. The therapeutically effective amount can be ascertained by measuring relevant physiological effects. The exact amount required varies from subject to subject, depending on the age, weight, and general condition of the subject, the severity of the condition being treated, the judgment of the clinician, and the like. An appropriate "effective amount" in any individual case can be determined by one of ordinary skill in the art using routine experimentation.

[0058] The term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" refers to a material that is suitable for drug administration to an individual along with an active agent without causing undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition.

[0059] The term "subject" as used herein refers to any animal (*e.g.*, a mammal), including, but not limited to, humans, non-human primates, canines, felines, rodents, and the like, which is to be the recipient of a particular treatment. A subject can be a human. A subject can have a particular disease or condition.

[0060] Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 *etc.*, as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

[0061] Exemplary genes and polypeptides are described herein with reference to GenBank numbers, GI numbers and/or SEQ ID NOs. It is understood that one skilled in the art can readily identify homologous sequences by reference to sequence sources, including but not limited to GenBank (ncbi.nlm.nih.gov/genbank/) and EMBL (embl.org/).

5.2 Pharmaceutical compositions

[0062] Provided herein are pharmaceutical compositions comprising an anti-OX40 antibody or antigen-binding fragment thereof at a high concentration (*e.g.*, at least 80 mg/mL) and one or more pharmaceutically acceptable or physiologically acceptable diluents, carriers or excipients. In some embodiments, the antibody and an additional agent(s) are present in a therapeutically acceptable amount. The pharmaceutical compositions can be used in accordance with the methods and uses provided herein. Thus, for example, the pharmaceutical compositions can be administered *ex vivo* or *in vivo* to a subject in order to practice treatment methods and uses provided herein. Pharmaceutical compositions provided herein can be formulated to be compatible with the intended method or route of administration; exemplary routes of administration are set forth herein.

[0063] Provided herein are pharmaceutical compositions comprising an anti-OX40 antibody or antigen-binding fragment thereof at a high concentration. The anti-OX40 antibodies or antigen-binding fragments can be any anti-OX40 antibody or antigen-binding fragment disclosed herein. For illustrative purposes, in some embodiments, pharmaceutical compositions provided herein comprise an anti-OX40 antibody or antigen-binding fragment thereof antibody comprising (1) a light chain variable region (VL) comprising VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:1, 2, and 3, respectively; and (2) a heavy chain variable region (VH) comprising VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of SEQ ID NOs:4, 5 and 6, respectively. In some embodiments, pharmaceutical compositions provided herein

comprise an anti-OX40 antibody or antigen-binding fragment thereof antibody comprising a VL and a VH that have the amino acid sequences of SEQ ID NOs:7 and 8, respectively.

[0064] In some embodiments, the pharmaceutical compositions provided herein comprise an anti-OX40 antibody or antigen-binding fragment at a concentration ranging from 80 to 180 mg/mL. In some embodiments, the pharmaceutical compositions provided herein comprise an anti-OX40 antibody or antigen-binding fragment at a concentration ranging from 100 to 180 mg/mL, from 120 to 180 mg/mL, from 140 to 180 mg/mL, from 100 to 160 mg/mL, from 120 to 160 mg/mL, from 140 to 160 mg/mL. In some embodiments, the pharmaceutical compositions provided herein comprise an anti-OX40 antibody or antigen-binding fragment at a concentration ranging from 140 to 160 mg/mL. In some embodiments, the pharmaceutical compositions provided herein comprise an anti-OX40 antibody or antigen-binding fragment at a concentration of about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 110 mg/mL, about 120 mg/mL, about 130 mg/mL, about 140 mg/mL, about 150 mg/mL, about 160 mg/mL, about 170 mg/mL, or about 180 mg/mL. In some embodiments, the pharmaceutical compositions provided herein comprise an anti-OX40 antibody or antigen-binding fragment at a concentration of about 100 mg/mL. In some embodiments, the pharmaceutical compositions provided herein comprise an anti-OX40 antibody or antigen-binding fragment at a concentration of about 120 mg/mL. In some embodiments, the pharmaceutical compositions provided herein comprise an anti-OX40 antibody or antigen-binding fragment at a concentration of about 140 mg/mL. In some embodiments, the pharmaceutical compositions provided herein comprise an anti-OX40 antibody or antigen-binding fragment at a concentration of about 150 mg/mL. In some embodiments, the pharmaceutical compositions provided herein comprise an anti-OX40 antibody or antigen-binding fragment at a concentration of about 160 mg/mL.

[0065] In some embodiments, pharmaceutical compositions provided herein comprise a buffer, a stabilizer and a surfactant. In some embodiments, pharmaceutical compositions provided herein comprise a glutamic/histidine buffer, proline, and polysorbate 80.

[0066] In some embodiments, pharmaceutical compositions provided herein comprise glutamic/histidine buffer. In some embodiments, the concentration of glutamic/histidine buffer useful in the pharmaceutical compositions provided herein ranges from 5 to 50 mM. In some embodiment, the concentration of the glutamic/histidine buffer ranges from 10 to 50 mM. In some embodiment, the concentration of the glutamic/histidine buffer ranges from 10 to 40 mM. In some embodiment, the concentration of the glutamic/histidine buffer ranges from 10 to 30 mM. In some embodiment, the concentration of the glutamic/histidine buffer ranges from 15 to 25 mM. In some embodiment, the concentration of the glutamic/histidine buffer is about 5 mM, about 10 mM, about 15 mM, about

20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, or about 50 mM. In some embodiment, the concentration of the glutamic/histidine buffer is about 10 mM. In some embodiment, the concentration of the glutamic/histidine buffer is about 15 mM. In some embodiment, the concentration of the glutamic/histidine buffer is about 20 mM. In some embodiment, the concentration of the glutamic/histidine buffer is about 25 mM. In some embodiment, the concentration of the glutamic/histidine buffer is about 30 mM.

[0067] As used herein and understood in the art, the concentration of the glutamic/histidine buffer refers to the combined molarities of L-glutamic acid and L-histidine. The ratio of L-glutamic acid to L-histidine can be adjusted by a person of ordinary skill in the art to reach the desired pH. In some embodiments, the ratio of L-glutamic acid to L-histidine can be about 1:1.

[0068] In some embodiments, the pharmaceutical compositions provided herein have a pH ranging from 4.8 to 6.8. In some embodiments, the pharmaceutical compositions provided herein have a pH ranging from 5.0 to 6.5. In some embodiments, the pharmaceutical compositions provided herein have a pH ranging from 5.0 to 6.0. In some embodiments, the pharmaceutical compositions provided herein have a pH ranging from 5.0 to 5.8. In some embodiments, the pharmaceutical compositions provided herein have a pH ranging from 5.0 to 5.6. In some embodiments, the pharmaceutical compositions provided herein have a pH ranging from 5.1 to 5.6. In some embodiments, the pH of the pharmaceutical composition is about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, or about 6.0. In some embodiments, the pH of the pharmaceutical composition is about 5.0. In some embodiments, the pH of the pharmaceutical composition is about 5.3. In some embodiments, the pH of the pharmaceutical composition is about 5.4. The pH can be measured at room temperature (*e.g.*, about 18-26 °C)

[0069] In some embodiments, pharmaceutical compositions provided herein comprise proline. In some embodiments, the concentration of proline useful in the pharmaceutical compositions provided herein ranges from 1.0 to 10.0% (w/v), from 1.0 to 9.0% (w/v), from 1.0 to 8.0% (w/v), from 1.0 to 7.0% (w/v), from 1.0 to 6.0% (w/v), from 1.0 to 5.0% (w/v), from 1.0 to 4.0% (w/v), from 1.0 to 3.0% (w/v), from 2.0 to 10.0% (w/v), from 2.0 to 9.0% (w/v), from 2.0 to 8.0% (w/v), from 2.0 to 7.0% (w/v), from 2.0 to 6.0% (w/v), from 2.0 to 5.0% (w/v), from 2.0 to 4.0% (w/v), or from 2.0 to 3.0% (w/v). In some embodiments, the concentration of proline ranges from 1.0 to 5.0% (w/v). In some embodiments, the concentration of proline ranges from 1.0 to 4.0% (w/v). In some embodiments, the concentration of proline ranges from 1.0 to 3.0% (w/v). In some embodiments, the concentration of proline ranges from 2.0 to 5.0% (w/v). In some embodiments, the concentration of proline ranges from 2.0 to 4.0% (w/v). In some embodiments, the concentration of proline ranges

from 2.0 to 3.0% (w/v). In some embodiments, the concentration of proline is about 1.0% (w/v), about 1.5% (w/v), about 2.0% (w/v), about 2.5% (w/v), about 3.0% (w/v), about 3.5% (w/v), about 4.0% (w/v), about 4.5% (w/v), about 5.0% (w/v), about 6.0% (w/v), about 7.0% (w/v), or about 8.0% (w/v). In some embodiments, the concentration of proline is about 1.5% (w/v). In some embodiments, the concentration of proline is about 2.0 % (w/v). In some embodiments, the concentration of proline is about 2.5% (w/v). In some embodiments, the concentration of proline is about 2.8% (w/v). In some embodiments, the concentration of proline is about 3.0% (w/v). In some embodiments, the concentration of proline is about 3.2% (w/v). In some embodiments, the concentration of proline is about 3.5% (w/v).

[0070] In some embodiments, pharmaceutical compositions provided herein comprise polysorbate 80. In some embodiments, the concentration of polysorbate 80 useful in the pharmaceutical compositions provided herein ranges from 0.001 to 0.1% (w/v), 0.0025 to 0.075% (w/v), from 0.005 to 0.05% (w/v), from 0.0075 to 0.05% (w/v), or from 0.01 to 0.05% (w/v). In some embodiments, the concentration of polysorbate 80 ranges from 0.005 to 0.05 (w/v). In some embodiments, the concentration of polysorbate 80 ranges from 0.01 to 0.03% (w/v). In some embodiments, the concentration of polysorbate 80 is about 0.005% (w/v), about 0.01% (w/v), about 0.015% (w/v), about 0.02% (w/v), about 0.025% (w/v), about 0.03% (w/v), about 0.035% (w/v), about 0.04% (w/v), about 0.045% (w/v), or about 0.05% (w/v). In some embodiments, the concentration of polysorbate 80 is about 0.005% (w/v). In some embodiments, the concentration of polysorbate 80 is about 0.01% (w/v). In some embodiments, the concentration of polysorbate 80 is about 0.02% (w/v). In some embodiments, the concentration of polysorbate 80 is about 0.03% (w/v).

[0071] In some embodiments, pharmaceutical compositions provided herein comprise 10-30 mM glutamic/histidine buffer, 2.5-3.0% (w/v) proline, and 0.01-0.03% (w/v) polysorbate 80. In some embodiments, pharmaceutical compositions provided herein comprise about 20 mM glutamic/histidine buffer, 2.8% (w/v) proline, and 0.01-0.03% (w/v) polysorbate 80. In some embodiments, pharmaceutical compositions provided herein have a pH ranging from 5.1 to 5.6. In some embodiments, pharmaceutical composition provided herein comprise about 20 mM glutamic/histidine buffer, about 2.8% (w/v) proline, and about 0.02% (w/v) polysorbate 80. In some embodiments, pharmaceutical compositions provided herein have a pH of about 5.3.

[0072] Pharmaceutical compositions provided herein can have low viscosity, which is desirable for, *e.g.*, subcutaneous injection. In some embodiments, the viscosity of the pharmaceutical compositions provided herein is no more than 20 cP, no more than 18 cP, no more than 16 cP, no more than 14 cP, no more than 12 cP, or no more than 10 cP. In some embodiments, the viscosity of the

pharmaceutical compositions provided herein is no more than 20 cP. In some embodiments, the viscosity of the pharmaceutical compositions provided herein is no more than 16 cP. In some embodiments, the viscosity of the pharmaceutical compositions provided herein is no more than 12 cP. In some embodiments, the viscosity of the pharmaceutical compositions provided herein ranges from 4 to 20 cP, from 6 to 20 cP, from 8 to 20 cP, from 10 to 20 cP, or from 12 to 20 cP. In some embodiments, the viscosity of the pharmaceutical compositions provided herein ranges from 4 to 15 cP, from 6 to 15 cP, from 8 to 15 cP, from 10 to 15 cP, or from 12 to 15 cP. In some embodiments, the viscosity of the pharmaceutical compositions provided herein ranges from 4 to 20 cP. In some embodiments, the viscosity of the pharmaceutical compositions provided herein ranges from 10 to 20 cP. In some embodiments, the viscosity of the pharmaceutical compositions provided herein ranges from 4 to 15 cP. In some embodiments, the viscosity of the pharmaceutical compositions provided herein ranges from 10 to 15 cP. In some embodiments, the viscosity of the pharmaceutical compositions provided herein is about 20 cP, about 18 cP, about 16 cP, about 14 cP, about 12 cP, or about 10 cP. In some embodiments, the viscosity of the pharmaceutical compositions provided herein is about 20 cP. In some embodiments, the viscosity of the pharmaceutical compositions provided herein is about 16 cP. In some embodiments, the viscosity of the pharmaceutical compositions provided herein is about 12 cP.

[0073] In some embodiments, the osmotic pressure of the pharmaceutical compositions provided herein ranges from 200 to 500 mOsmol/kg, from 250 to 500 mOsmol/kg, from 300 to 500 mOsmol/kg, from 200 to 400 mOsmol/kg, from 220 to 400 mOsmol/kg, from 250 to 400 mOsmol/kg, from 280 to 400 mOsmol/kg, or from 300 to 400 mOsmol/kg. In some embodiments, the osmotic pressure of the pharmaceutical composition ranges from 200 to 400 mOsmol/kg. In some embodiments, the osmotic pressure of the pharmaceutical composition ranges from 250 to 400 mOsmol/kg. In some embodiments, the osmotic pressure of the pharmaceutical composition ranges from 300 to 400 mOsmol/kg. In some embodiments, the osmotic pressure of the pharmaceutical composition ranges from 303 to 403 mOsmol/kg. In some embodiments, the osmotic pressure of the pharmaceutical compositions provided herein is about 200 mOsmol/kg, about 250 mOsmol/kg, about 300 mOsmol/kg, about 350 mOsmol/kg, about 380 mOsmol/kg or about 400 mOsmol/kg. In some embodiments, the osmotic pressure is about 300 mOsmol/kg. In some embodiments, the osmotic pressure is about 350 mOsmol/kg.

[0074] In some embodiments of the pharmaceutical compositions provided herein, the purity of the antibody or antigen-binding fragment as measured by Size Exclusion Chromatography (SEC) is at least 95%, at least 95.5%, at least 96%, at least 96.5%, at least 97%, at least 97.5%, at least 98%, at

least 98.5%, or at least 99%. In some embodiments of the pharmaceutical compositions provided herein, the purity of the antibody or antigen-binding fragment as measured by SEC is at least 95%. In some embodiments of the pharmaceutical compositions provided herein, the purity of the antibody or antigen-binding fragment as measured by SEC is at least 98.5%.

[0075] In some embodiments, the pharmaceutical compositions provided herein comprise no more than 5%, no more than 4%, no more than 3%, no more than 2%, or no more than 1% High Molecular Weight Substance (HMWS) as measured by SEC. In some embodiments, the pharmaceutical compositions provided herein comprise no more than 5% HMWS as measured by SEC. In some embodiments, the pharmaceutical compositions provided herein comprise no more than 2% HMWS as measured by SEC.

[0076] In some embodiments of the pharmaceutical compositions provided herein, the purity of the antibody or antigen-binding fragment as measured by Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS) is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, or at least 99%. In some embodiments of the pharmaceutical compositions provided herein, the purity of the antibody or antigen-binding fragment as measured by CE-SDS is at least 90%. In some embodiments of the pharmaceutical compositions provided herein, the purity of the antibody or antigen-binding fragment as measured by CE-SDS is at least 97%.

[0077] In some embodiments, the pharmaceutical compositions provided herein comprise no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%, no more than 2%, or no more than 1% Total Low Molecular Weight Substance (LMWS) as measured by CE-SDS. In some embodiments, the pharmaceutical compositions provided herein comprise no more than 10% Total LMWS as measured by CE-SDS. In some embodiments, the pharmaceutical compositions provided herein comprise no more than 2% Total LMWS as measured by CE-SDS. In some embodiments, the Total LMWS% is measured by CE-SDS (Reduced). In some embodiments, the Total LMWS% is measured by CE-SDS (Non-reduced).

[0078] In some embodiments of the pharmaceutical compositions provided herein, the charge variants of the antibody or antigen-binding fragment as measured by Cation Exchange chromatography (CEX) is no more than 60%, no more than 55%, no more than 50%, no more than 45%, or no more than 40%, no more than 35% or no more than 30%. In some embodiments of the pharmaceutical compositions provided herein, the charge variants of the antibody or antigen-binding fragment as measured by CEX is no more than 60%. In some embodiments of the pharmaceutical

compositions provided herein, the charge variants of the antibody or antigen-binding fragment as measured by CEX is no more than 30%.

[0079] In some embodiments, the pharmaceutical compositions provided herein comprise no more than 40%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, or no more than 15% Acidic Peaks as measured by CEX. In some embodiments, the pharmaceutical compositions provided herein comprise no more than 40% Acidic Peaks as measured by CEX. In some embodiments, the pharmaceutical compositions provided herein comprise no more than 20% Acidic Peaks as measured by CEX.

[0080] In some embodiments, the pharmaceutical compositions provided herein comprise no more than 30%, no more than 25%, no more than 20%, no more than 15%, or no more than 10% Basic Peaks as measured by CEX. In some embodiments, the pharmaceutical compositions provided herein comprise no more than 30% Basic Peaks as measured by CEX. In some embodiments, the pharmaceutical compositions provided herein comprise no more than 15% Basic Peaks as measured by CEX.

[0081] In some embodiments, the pharmaceutical compositions are characterized as follows:

Items	**Ranges**
pH	5.0-5.6
Osmolarity	303-403 mOsmol/Kg
Protein Concentration	140.0-160.0 mg/mL
Charge Variants content (CEX)	
Main Peak	≥ 40.0%
Acidic Peaks	≤ 40.0%
Basic Peaks	≤ 30.0%
Purity by SEC	
Main Peak%	≥ 95.0%
HMWS%	≤ 5.0%
Purity by CE-SDS (Reduced)	
Main Peak%	≥ 90.0%
HMWS%	≤ 10.0%
Purity by CE-SDS (Non-Reduced)	
Main Peak%	≥ 90.0%
HMWS%	≤ 10.0%

Polysorbate80 (w/v)	0.010%-0.030%

[0082] In some embodiments, the pharmaceutical compositions provided herein are stable for long-term storage (*e.g.*, two weeks or longer). In some embodiments, the purity of the antibody or antigen-binding fragment is reduced by no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%, or no more than 2% after storage for a period of time that is at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11, at least months, at least 12 months, at least 18 months, or at least 24 month. The purity of the pharmaceutical compositions provided herein can be measured by various means available in the art. The purity can be measured by SEC. The purity can be measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC). The purity can be measured by CE-SDS. The purity can be measured by CE-SDS (Reduced). The purity can be measured by CE-SDS (Non-Reduced).

[0083] In some embodiments, the charge variants of the antibody or antigen-binding fragment are increased by no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5%, after storage for a period of time that is at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11, at least months, at least 12 months, at least 18 months, or at least 24 month. The charge variants of the pharmaceutical compositions provided herein can be measured by various means available in the art. The charge variants can be measured by CEX. The charge variants can be measured by Cation Exchange-High Performance Liquid Chromatography (CEX-HPLC).

[0084] The pharmaceutical composition can be stored at about -80°C, about -20°C, about 4°C, about 25°C or about 40°C. In some embodiments, the purity of the antibody or antigen-binding fragment is reduced by no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%, or no more than 2% after storage at about 4°C for a period of time that is at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11, at least months, at least 12 months, at least 18 months, or at least 24 month. In some embodiments, the purity of the antibody or antigen-binding fragment is reduced by no more than 5% after storage at about 4°C for at least 3

months. In some embodiments, the purity of the antibody or antigen-binding fragment is reduced by no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%, or no more than 2% after storage at about 25°C for a period of time that is at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11, at least months, at least 12 months, at least 18 months, or at least 24 month. In some embodiments, the purity of the antibody or antigen-binding fragment is reduced by no more than 5% after storage at about 25°C for at least 3 months.

[0085] In some embodiments, the charge variants of the antibody or antigen-binding fragment are increased by no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5% after storage at about 4°C for a period of time that is at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11, at least months, at least 12 months, at least 18 months, or at least 24 month. In some embodiments, the charge variants of the antibody or antigen-binding fragment are increased by no more than 5% after storage at about 4°C for at least 3 months. In some embodiments, the charge variants of the antibody or antigen-binding fragment are increased by no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5% after storage at about 25°C for a period of time that is at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11, at least months, at least 12 months, at least 18 months, or at least 24 month. In some embodiments, the charge variants of the antibody or antigen-binding fragment are increased by no more than 10% after storage at about 25°C for at least 3 months.

[0086] The pharmaceutical compositions can be stored upright or inverted. In some embodiments, the pharmaceutical compositions provided herein are stable when stored upright. In some embodiments, the pharmaceutical compositions are stable when stored inverted. In some embodiments, the pharmaceutical compositions provided herein with agitation (*e.g.*, shaking).

[0087] The pharmaceutical compositions provided herein are stable upon lyophilization. In some embodiments, the pharmaceutical compositions provided herein are stable after at least 1, at least 2, at least 3, at least 4, or at least 5 freeze-thaw cycles. In some embodiments, the purity of the antibody or antigen-binding fragment is reduced by no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%,

or no more than 2% after at least 1, at least 2, at least 3, at least 4, or at least 5 freeze-thaw cycles. In some embodiments, the purity of the antibody or antigen-binding fragment is reduced by no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%, or no more than 2% after at least 3 freeze-thaw cycles. In some embodiments, the purity of the antibody or antigen-binding fragment is reduced by no more than 5% after at least 3 freeze-thaw cycles. The purity of the pharmaceutical compositions provided herein can be measured by various means available in the art. The purity can be measured by SEC. The purity can be measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC). The purity can be measured by CE-SDS. The purity can be measured by CE-SDS (Reduced). The purity can be measured by CE-SDS (Non-Reduced).

[0088] In some embodiments, the charge variants of the antibody or antigen-binding fragment are increased by no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5% after at least 1, at least 2, at least 3, at least 4, or at least 5 freeze-thaw cycles. In some embodiments, the charge variants of the antibody or antigen-binding fragment are increased by no more than 20% after at least 3 freeze-thaw cycles. In some embodiments, the charge variants of the antibody or antigen-binding fragment are increased by no more than 20% after at least 5 freeze-thaw cycles. The charge variants of the pharmaceutical compositions provided herein can be measured by various means available in the art. The charge variants can be measured by CEX. The charge variants can be measured by Cation Exchange-High Performance Liquid Chromatography (CEX-HPLC).

[0089] Pharmaceutical compositions provided herein can be stored in a sterile vial as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. In some embodiments, the pharmaceutical compositions provided herein are in liquid form. In some embodiments, the pharmaceutical compositions provided herein are lyophilized.

[0090] In some embodiments, pharmaceutical compositions provided herein can further include antimicrobial agent or preservative. Suitable antimicrobial agents or preservatives include, but are not limited to, phenols, cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoates, thimerosal, benzalkonium chloride (*e.g.*, benzethonium chloride), methyl- and propyl-parabens, and sorbic acid. In some embodiments, pharmaceutical compositions provided herein can further include an antioxidant. Suitable antioxidants are those as described herein, including bisulfite and sodium metabisulfite. In some embodiments, pharmaceutical compositions provided herein can further include a local anesthetic. Suitable local anesthetics include, but are not limited to, procaine hydrochloride.

[0091] In some embodiments, the pharmaceutical compositions provided herein can be formulated for single or multiple dosage administration. The single dosage formulations are packaged in an ampoule, a vial, or a syringe. The multiple dosage parenteral formulations can contain an antimicrobial agent at bacteriostatic or fungistatic concentrations. All parenteral formulations must be sterile, as known and practiced in the art.

[0092] In some embodiments, pharmaceutical compositions provided herein can be stored either in a ready to use form, a lyophilized form requiring reconstitution prior to use, a liquid form requiring dilution prior to use, or other acceptable form. In some embodiments, a pharmaceutical composition is provided in a single-use container (*e.g.*, a single-use vial, ampoule, syringe, or autoinjector (similar to, *e.g.*, an EpiPen®)), whereas a multi-use container (*e.g.*, a multi-use vial) is provided in other embodiments.

[0093] In some embodiments, the pharmaceutical compositions are provided as ready-to-use sterile solutions. In some embodiments, the pharmaceutical compositions are provided as sterile dry soluble products, including lyophilized powders and hypodermic tablets, to be reconstituted with a vehicle prior to use. In yet another embodiment, the pharmaceutical compositions are provided as ready-to-use sterile suspensions. In yet another embodiment, the pharmaceutical compositions are provided as sterile dry insoluble products to be reconstituted with a vehicle prior to use. In still another embodiment, the pharmaceutical compositions are provided as ready-to-use sterile emulsions.

5.3 Anti-OX40 antibodies and antigen-binding fragments

[0094] Provided herein are pharmaceutical compositions having an anti-OX40 antibody or antigen-binding fragment thereof. The anti-OX40 antibody to be used in the methods described herein can be any anti-OX40 antibody disclosed in WO2021209019A1, which is hereby incorporated by reference in its entirety.

[0095] In some embodiments, the anti-OX40 antibodies or antigen-binding fragments used in methods disclosed herein are antagonist antibodies that have a blocking activity for OX40-mediated signal transduction. In some embodiments, the anti-OX40 antibodies or antigen-binding fragments used in methods disclosed herein are monoclonal antibodies or antigen-binding fragments. Monoclonal antibodies can be prepared by any method known to those of skill in the art. In some embodiments, a monoclonal antibody is modified using recombinant DNA technology to generate alternative antibodies. In some embodiments, the constant domains of the light chain and heavy chain of a mouse monoclonal antibody are replaced with the constant regions of a human antibody to generate a chimeric antibody. In some embodiments, the constant regions are truncated or removed to generate a desired antibody fragment of a monoclonal antibody. In some embodiments, site-

directed or high-density mutagenesis of the variable region(s) is used to optimize specificity and/or affinity of a monoclonal antibody.

[0096] In some embodiments, the anti-OX40 antibodies or antigen-binding fragments provided herein are isolated. In some embodiments, the anti-OX40 antibodies or antigen-binding fragments provided herein are substantially pure.

[0097] In some embodiments, provided herein are anti-OX40 antibodies. In some embodiments, the antibody is an IgA, IgD, IgE, IgG, or IgM antibody. In some embodiments, the antibody is an IgA antibody. In some embodiments, the antibody is an IgD antibody. In some embodiments, the antibody is an IgE antibody. In some embodiments, the antibody is an IgG antibody. In some embodiments, the antibody is an IgM antibody. In some embodiments, the antibodies provided herein can be an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody. In some embodiments, the antibody is an IgG1 antibody. In some embodiments, the antibody is an IgG2 antibody. In some embodiments, the antibody is an IgG3 antibody. In some embodiments, the antibody is an IgG4 antibody.

[0098] In some embodiments, provided herein are antigen-binding fragments of an anti-OX40 antibody. In some embodiments, antigen-binding fragments provided herein can be a single domain antibody (sdAb), a heavy chain antibody (HCAb), a Fab, a Fab', a $F(ab')_2$, a Fv, a single-chain variable fragment (scFv), or a $(scFv)_2$. In some embodiments, the antigen-binding fragment of an anti-OX40 antibody is a single domain antibody (sdAb). In some embodiments, the antigen-binding fragment of an anti-OX40 antibody is a heavy chain antibody (HCAb). In some embodiments, the antigen-binding fragment of an anti-OX40 antibody is a Fab. In some embodiments, the antigen-binding fragment of an anti-OX40 antibody is a Fab'. In some embodiments, the antigen-binding fragment of an anti-OX40 antibody is a $F(ab')_2$. In some embodiments, the antigen-binding fragment of an anti-OX40 antibody is a Fv. In some embodiments, the antigen-binding fragment of an anti-OX40 antibody is a scFv. In some embodiments, the antigen-binding fragment of an anti-OX40 antibody is a disulfide-linked scFv [$(scFv)_2$]. In some embodiments, the antigen-binding fragment of an anti-OX40 antibody is a diabody (dAb).

[0099] In some embodiments, the anti-OX40 antibodies or antigen-binding fragments provided herein comprise recombinant antibodies or antigen-binding fragments. In some embodiments, the anti-OX40 antibodies or antigen-binding fragments provided herein comprise monoclonal antibodies or antigen-binding fragments. In some embodiments, the anti-OX40 antibodies or antigen-binding fragments provided herein comprise polyclonal antibodies or antigen-binding fragments. In some embodiments, the anti-OX40 antibodies or antigen-binding fragments provided herein comprise

camelid (*e.g.*, camels, dromedary and llamas) antibodies or antigen-binding fragments. In some embodiments, the anti-OX40 antibodies or antigen-binding fragments provided herein comprise chimeric antibodies or antigen-binding fragments. In some embodiments, the anti-OX40 antibodies or antigen-binding fragments provided herein comprise humanized antibodies or antigen-binding fragments. In some embodiments, the anti-OX40 antibodies or antigen-binding fragments provided herein comprise human antibodies or antigen-binding fragments. In some embodiments, provided herein are anti-OX40 humanized IgG1 antibodies.

[0100] In some embodiments, provided herein is an anti-OX40 antibody with the sequence features described below. The specific CDR sequences defined herein are generally based on Kabat definition. However, it is understood that a general reference to a heavy chain CDR or CDRs and/or a light chain CDR or CDRs of a specific antibody encompasses all CDR definitions as known to those of skill in the art.

[0101] In some embodiments, anti-OX40 antibodies or antigen-binding fragments provided herein comprise one, two, three, four, five, and/or six CDRs of any one of the antibodies described herein. In some embodiments, anti-OX40 antibodies or antigen-binding fragments provided herein comprise a light chain variable region (VL) comprising one, two, and/or three, light chain CDRs (VL CDRs) from Table 1. In some embodiments, anti-OX40 antibodies or antigen-binding fragments provided herein comprise a heavy chain variable region (VH) comprising one, two, and/or three heavy chain CDRs (VH CDRs) from Table 1. In some embodiments, anti-OX40 antibodies or antigen-binding fragments provided herein comprise one, two, and/or three VL CDRs and one, two, and/or three VH CDRs from Table 1.

Table 1: CDRs

VL CDR1	**VL CDR2**	**VL CDR3**
RASESVDSSGNSFMH (SEQ ID NO:1)	RASNLES (SEQ ID NO:2)	QQSNEDPWT (SEQ ID NO:3)
VH CDR1	**VH CDR2**	**VH CDR3**
SYWVD (SEQ ID NO:4)	NIYPSDSETHYNQKFKD (SEQ ID NO:5)	SYGYYGTWFAY (SEQ ID NO:6)

[0102] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to OX40, comprising, (a) a VL comprising VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:1, 2, and 3, respectively; or a variant thereof having up to about 3, about 5, about 8, about 10, about 12, or about 15 amino acid substitutions, additions, and/or deletions in the VL CDRs; and/or (b) a VH comprising VH CDR1, VH CDR2, and

VH CDR3 having the amino acid sequences of SEQ ID NOs:4, 5 and 6, respectively; or a variant thereof having up to having up to about 3, about 5, about 8, about 10, about 12, or about 15 amino acid substitutions, additions, and/or deletions in the VH CDRs.

Table 2: VLs and VHs

Ab domain	Sequence
VL1	DIVMTQSPDSLAVSLGERATINCRASESV**DS**SG*N**S***F**M**HWYQQKPGQPP KLLIYRASNLESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSNE DP**W**TFGGGTKLEIK (SEQ ID NO:7)
VL2	DIVMTQSPDSLAVSLGERATINCRASESVDSSGNSFMHWYQQKPGQPP KLLIYRASNLESGIPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQQSNE DPWTFGGGTKLEIK (SEQ ID NO:9)
VL3	DIVMTQSPDSLAVSLGERATINCRASESVDSSGNSFMHWYQQKPGQPP KLLIYRASNLESGIPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSNED PWTFGGGTKLEIK (SEQ ID NO:10)
VH1	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSY**W**VDWVRQAPGQGLE WMGNIYPS**DS**ETHYNQKFKDRVTMTRDTSTSTVYMELSSLRSEDTAV YYCARSYGYYGT**W**FAYWGQGTLVTVSS (SEQ ID NO:8)
VH2	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWVDWVRQAPGQGLE WIGNIYPSDSETHYNQKFKDRVTMTVDTSTSTVYMELSSLRSEDTAVY YCARSYGYYGTWFAYWGQGTLVTVSS (SEQ ID NO:11)
VH3	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWVDWVRQAPGQGLE WIGNIYPSDSETHYNQKFKDRVTMTVDTSTSTAYMELSSLRSEDSAVY YCARSYGYYGTWFAYWGQGTLVTVSS (SEQ ID NO:12)

[0103] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to OX40 comprising a VL having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:7. In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to OX40 comprising a VH having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:8.

[0104] In some embodiments, the anti-OX40 antibodies described herein comprise a VL having the amino acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:10. In some embodiments, the anti-OX40 antibodies described herein comprise a VH having the amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:11 and SEQ ID NO:12. In some embodiments, the anti-OX40 antibodies described herein comprise a VL having the amino acid sequence of SEQ ID NO:7. In some embodiments, the anti-OX40 antibodies described herein comprise a VL having the amino acid sequence of SEQ ID NO:9. In some embodiments, the anti-OX40 antibodies described herein comprise a VL having the amino acid sequence of SEQ ID NO:10. In some embodiments, the anti-OX40 antibodies described herein comprise a VH having the amino acid sequence of SEQ ID NO:8. In some embodiments, the anti-OX40 antibodies described herein comprise a VH having the amino acid sequence of SEQ ID NO:11. In some embodiments, the anti-OX40 antibodies described herein comprise a VH having the amino acid sequence of SEQ ID NO:12. In some embodiments, the anti-OX40 antibodies described herein comprise a VL and a VH having the amino acid sequences of SEQ ID NOs:7 and 8, respectively. In some embodiments, the anti-OX40 antibodies described herein comprise a VL and a VH having the amino acid sequences of SEQ ID NOs:7 and 11, respectively. In some embodiments, the anti-OX40 antibodies described herein comprise a VL and a VH having the amino acid sequences of SEQ ID NOs:7 and 12, respectively. In some embodiments, the anti-OX40 antibodies described herein comprise a VL and a VH having the amino acid sequences of SEQ ID NOs:9 and 8, respectively. In some embodiments, the anti-OX40 antibodies described herein comprise a VL and a VH having the amino acid sequences of SEQ ID NOs:9 and 11, respectively. In some embodiments, the anti-OX40 antibodies described herein comprise a VL and a VH having the amino acid sequences of SEQ ID NOs:9 and 12, respectively. In some embodiments, the anti-OX40 antibodies described herein comprise a VL and a VH having the amino acid sequences of SEQ ID NOs:10 and 8, respectively. In some embodiments, the anti-OX40 antibodies described herein comprise a VL and a VH having the amino acid sequences of SEQ ID NOs:10 and 11, respectively. In some embodiments, the anti-OX40 antibodies described herein comprise a VL and a VH having the amino acid sequences of SEQ ID NOs:10 and 12, respectively.

[0105] In some embodiments, anti-OX40 antibodies provided herein are IgA, IgD, IgE, IgG, or IgM antibodies. In some embodiments, the antibody is an IgA antibody. In some embodiments, the antibody is an IgD antibody. In some embodiments, the antibody is an IgE antibody. In some embodiments, the antibody is an IgG antibody. In some embodiments, the antibody is an IgM antibody. In some embodiments, the antibodies provided herein can be an IgG1 antibody, an IgG2

antibody, an IgG3 antibody, or an IgG4 antibody. In some embodiments, the antibody is an IgG1 antibody. In some embodiments, the antibody is an IgG2 antibody. In some embodiments, the antibody is an IgG3 antibody. In some embodiments, the antibody is an IgG4 antibody.

[0106] In some embodiments, anti-OX40 antibodies provided herein comprise a light chain and a heavy chain. The light chain can comprise a light chain constant domain (CL) and a light chain variable domain (VL). The heavy chain can comprise a heavy chain variable domain (VH) and a heavy chain constant domain (CH). The VL/VH can be any VL/VH disclosed herein. In some embodiments, the light chain constant region (CL) has at least 85%, at least 90%, at least 95% or at least 99% sequence identity to kappa CL kappa CL (Cκ; SEQ ID NO:13). In some embodiments, the light chain constant region (CL) is kappa CL (Cκ; SEQ ID NO:13). In some embodiments, the light chain constant region (CL) has at least 85%, at least 90%, at least 95% or at least 99% sequence identity to lambda CL (Cλ; SEQ ID NO:14). In some embodiments, the light chain constant region (CL) is lambda CL (Cλ; SEQ ID NO:14).

[0107] In some embodiments, the heavy chain can comprise a heavy chain constant domain (CH) from human IgA, IgD, IgE, IgM, or IgG. In some embodiments, the heavy chain can comprise a heavy chain constant domain (CH) having at least 85%, at least 90%, at least 95% or at least 99% sequence identity to the CH of human IgG1 (*e.g.*, SEQ ID NO:15). In some embodiments, the heavy chain can comprise a heavy chain CH having at least 85%, at least 90%, at least 95% or at least 99% sequence identity to the CH of human IgG2 (*e.g.*, SEQ ID NO:16). In some embodiments, the heavy chain can comprise a heavy chain CH having at least 85%, at least 90%, at least 95% or at least 99% sequence identity to the CH of human IgG3 (*e.g.*, SEQ ID NO:17). In some embodiments, the heavy chain can comprise a heavy chain CH having at least 85%, at least 90%, at least 95% or at least 99% sequence identity to the CH of human IgG4 (*e.g.*, SEQ ID NO:18). In some embodiments, the heavy chain can comprise a heavy chain constant domain (CH) from human IgG1 (*e.g.*, SEQ ID NO:15). In some embodiments, the heavy chain can comprise a heavy chain constant domain (CH) from human IgG2 (*e.g.*, SEQ ID NO:16). In some embodiments, the heavy chain can comprise a heavy chain constant domain (CH) from human IgG3 (*e.g.*, SEQ ID NO:17). In some embodiments, the heavy chain can comprise a heavy chain constant domain (CH) from human IgG4 (*e.g.*, SEQ ID NO:18). Expressly contemplated here are any and all combinations of the VL/VH pairs disclosed herein that specifically bind to OX40 (*e.g.*, human OX40) and the CL/CH disclosed herein or otherwise known in the art.

Light Chain Constant Region	Sequences
kappa CL (Cκ)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC (SEQ ID NO:13)
lambda CL (Cλ)	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKAD SSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVT HEGSTVEKTVAPTECS (SEQ ID NO:14)

Heavy Chain Constant Region	Sequences
IgG1 CH	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO:15)
IgG2 CH	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNT KVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVV SVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKT TPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK (SEQ ID NO:16)
IgG3 CH	ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSN TKVDKRVELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDT PPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNST FRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPEN NYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHN RFTQKSLSLSPGK (SEQ ID NO:17)
IgG4 CH	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNT KVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT QKSLSLSLGK (SEQ ID NO:18)

[0108] The present disclosure further contemplates additional variants and equivalents that are substantially homologous to the antibodies described herein. In some embodiments, it is desirable to

improve the binding affinity of the antibody. In some embodiments, it is desirable to modulate biological properties of the antibody, including but not limited to, specificity, thermostability, expression level, effector function(s), glycosylation, immunogenicity, and/or solubility. Those skilled in the art would appreciate that amino acid changes may alter post-translational processes of an antibody, such as changing the number or position of glycosylation sites or altering membrane anchoring characteristics.

[0109] Variations can be a substitution, deletion, or insertion of one or more nucleotides encoding the antibody or polypeptide that results in a change in the amino acid sequence as compared with the native antibody or polypeptide sequence. In some embodiments, amino acid substitutions are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *e.g.*, conservative amino acid replacements. Insertions or deletions can be in the range of about 1 to 5 amino acids. In some embodiments, the substitution, deletion, or insertion includes less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the parent molecule. In some embodiments, variations in the amino acid sequence that are biologically useful and/or relevant can be determined by systematically making insertions, deletions, or substitutions in the sequence and testing the resulting variant proteins for activity as compared to the parent protein. In some embodiments, a variant comprises one to five amino acid substitutions, additions, and/or deletions in the parent antibody or antigen-binding fragment. In some embodiments, a variant comprises one to three amino acid substitutions, additions, and/or deletions in the parent antibody or antigen-binding fragment. In some embodiments, the amino acid substitution(s) is in a CDR of the antibody or antigen-binding fragment. In some embodiments, the amino acid substitution(s) is not in a CDR of the antibody or antigen-binding fragment. In some embodiments, the amino acid substitution(s) is in a framework region of the antibody or antigen-binding fragment. In some embodiments, the amino acid substitutions, additions, and/or deletions are conservative amino acid substitutions.

[0110] It is known in the art that the constant region(s) of an antibody mediates several effector functions and these effector functions can vary depending on the isotype of the antibody. For example, binding of the C1 component of complement to the Fc region of IgG or IgM antibodies (bound to antigen) activates the complement system. Activation of complement is important in the opsonization and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and can be involved in autoimmune hypersensitivity. In addition, the Fc

region of an antibody can bind a cell expressing a Fc receptor (FcR). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody-dependent cell cytotoxicity or ADCC), release of inflammatory mediators, placental transfer, and control of immunoglobulin production. In some embodiments, at least one or more of the constant regions has been modified or deleted in the anti-OX40 antibodies. In some embodiments, the antibodies comprise modifications to one or more of the three heavy chain constant regions (CH1, CH2 or CH3) and/or to the light chain constant region (CL). In some embodiments, the constant region modifications decrease or remove ADCC and/or CDC of the antibody. In some embodiments, the antibodies do not have one or more effector functions (*e.g.*, "effectorless" antibodies). In some embodiments, the antibodies do not bind an Fc receptor and/or complement factors. In some embodiments, the antibodies have no effector function(s). In some embodiments, the constant region is modified to eliminate disulfide linkages or oligosaccharide moieties. In some embodiments, the constant region is modified to add/substitute one or more amino acids to provide one or more cytotoxin, oligosaccharide, or carbohydrate attachment sites. In some embodiments, an anti-OX40 antibodies provided herein comprise a variant Fc region that is engineered with substitutions at specific amino acid positions as compared to a native Fc region.

[0111] In some embodiments, the anti-OX40 antibodies disclosed herein comprise modifications to reduce or eliminate their binding to FcR. In some embodiments, the anti-OX40 antibodies disclosed herein comprise modifications to reduce or eliminate their ADCC activities.

[0112] In some embodiments, the anti-OX40 antibodies described herein further comprise a Fc region variant. In some embodiments, the anti-OX40 antibodies described herein comprise an IgG1 heavy chain constant region that has an amino acid substitution at N297, numbered in accordance with EU index. In some embodiments, the substitution is N297A. The term "IgG1 N297A" refers to an Fc region variant of IgG1 that has the substitution of asparagine with alanine at position 297 relative to the parent polypeptide (*i.e.*, the wild-type Fc region of IgG1), where the number is in accordance with EU index. Expressly contemplated for inclusion into antibodies disclosed herein are heavy chain constant region (CH) of any immunoglobulin (*e.g.*, human IgG1) disclosed herein or otherwise known in the art with any combinations of mutations known in the art for reducing or eliminating ADCC and/or CDC of the antibodies.

[0113] The anti-OX40 antibodies or antigen-binding fragments of the present disclosure can be analyzed for their physical, chemical and/or biological properties by various methods known in the

art. In some embodiments, an anti-OX40 antibody is tested for its ability to bind to OX40 (*e.g.*, human OX40). In some embodiments, an anti-OX40 antibody is tested for its ability to bind an FcγR. Binding assays include, but are not limited to, BLI, SPR (*e.g.*, Biacore), ELISA, and FACS. In addition, antibodies can be evaluated for solubility, stability, thermostability, viscosity, expression levels, expression quality, and/or purification efficiency.

[0114] In some embodiments, the anti-OX40 antibodies described herein comprises a light chain having an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to SEQ ID NO:19. In some embodiments, the light chain can have the amino acid sequence of SEQ ID NO:19. In some embodiments, the anti-OX40 antibodies described herein comprises a heavy chain having an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:20. In some embodiments, the heavy chain can have the amino acid sequence of SEQ ID NO:20. The nucleic acid sequences encoding the light chain and heavy chain of the anti-OX40 antibodies described herein comprise SEQ ID NO:21 and SEQ ID NO:22 respectively.

Table 3: light chain (LC) and heavy chain (HC)

Ab chain	**Sequence**
LC	DIVMTQSPDSLAVSLGERATINCRASESVDSSGNSFMHWYQQKPGQPP KLLIYRASNLESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSNE DPWTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:19)
HC	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWVDWVRQAPGQGLE WMGNIYPSDSETHYNQKFKDRVTMTRDTSTSTVYMELSSLRSEDTAV YYCARSYGYYGTWFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:20)

[0115] In some embodiments, anti-OX40 antibodies described herein can comprise additional post translational modifications. In some embodiments, anti-OX40 antibodies described herein can be chemically modified naturally or by intervention. In some embodiments, the anti-OX40 antibodies disclosed herein can be modified with glycosylation, oxidation, acetylation, pegylation,

phosphorylation, amidation, deamidation, derivatization by known protecting/blocking groups, proteolytic cleavage, and/or linkage to a cellular ligand or other protein. Any of numerous chemical modifications can be carried out by known techniques. The anti-OX40 antibodies can comprise one or more analogs of an amino acid (including, for example, unnatural amino acids), as well as other modifications known in the art. For example, the anti-OX40 antibodies described herein can comprise glycosylation site, oxidation site and/or deamidation site in its VL and/or VH. In particular, for the anti-OX40 antibodies having a VL and a VH having the amino acid sequences of SEQ ID NOs:7 and 8, as set forth in Table 2, the potential oxidation sites are bolded, the potential deamidation sites are in italics and bolded, and the potential isomerization sites are underlined and bolded. Compared with the other two OX40 antagonists with reserved (telazorlimab) or enhanced (rocatinlimab) antibody-dependent cellular cytotoxicity (ADCC) function, the anti-OX40 antibodies described herein with modifications in the Fc region to remove glycosylation have abolished ADCC and minimized potential toxicities associated with T cell depletion.

[0116] The OX40 antibodies described herein can be prepared using any methods known in the art. Methods of antibody production are well-known in the art. *See* for example, in Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in:MONOCLONAL ANTIBODIES AND T-CELL HYBRIDOMAS 563 681 (Elsevier, N.Y., 1981), each of which is incorporated herein by reference in its entirety. In some embodiments, monoclonal antibodies are prepared using hybridoma methods known to one of skill in the art. In some embodiments, monoclonal antibodies are made using recombinant DNA techniques as known to one skilled in the art. For example, the polynucleotides encoding an antibody are isolated from mature B-cells or hybridoma cells, such as by RT-PCR using oligonucleotide primers that specifically amplify the genes encoding the heavy and light chains of the antibody, and their sequence is determined using standard techniques. The isolated polynucleotides encoding the heavy and light chains are then cloned into suitable expression vectors which produce the monoclonal antibodies when transfected into host cells such as *E. coli*, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin proteins.

[0117] Peptides can also be synthesized, in whole or in part, using chemical methods (*see, e.g.*, Caruthers (1980). *Nucleic Acids Res. Symp. Ser.* 215; Horn (1980); and Banga, A.K., THERAPEUTIC PEPTIDES AND PROTEINS, FORMULATION, PROCESSING AND DELIVERY SYSTEMS (1995) Technomic Publishing Co., Lancaster, PA). Peptide synthesis can be performed using various solid phase techniques (*see, e.g.*, Roberge, *Science* 269:202 (1995); Merrifield, *Methods. Enzymol.* 289:3 (1997)) and automated synthesis may be achieved, *e.g.*, using the ABI 431A Peptide Synthesizer (Perkin

Elmer) in accordance with the manufacturer's instructions. Peptides can also be synthesized using combinatorial methodologies. Synthetic residues and polypeptides can be synthesized using a variety of procedures and methodologies known in the art (*see*, *e.g.*, ORGANIC SYNTHESES COLLECTIVE VOLUMES, Gilman, *et al.* (Eds) John Wiley & Sons, Inc., NY). Modified peptides can be produced by chemical modification methods (see, for example, Belousov, *Nucleic Acids Res*. 25:3440 (1997); Frenkel, *Free Radic. Biol. Med*. 19:373 (1995); and Blommers, *Biochemistry* 33:7886 (1994)). Peptide sequence variations, derivatives, substitutions and modifications can also be made using methods such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR based mutagenesis. Site-directed mutagenesis (Carter *et al.*, *Nucl. Acids Res.*, 13:4331 (1986); Zoller *et al.*, *Nucl. Acids Res.* 10:6487 (1987)), cassette mutagenesis (Wells *et al.*, *Gene* 34:315 (1985)), restriction selection mutagenesis (Wells *et al.*, *Philos. Trans. R. Soc. London SerA* 317:415 (1986)) and other techniques can be performed on cloned DNA to produce invention peptide sequences, variants, fusions and chimeras, and variations, derivatives, substitutions and modifications thereof.

5.4 Methods of Preparation

[0118] The present disclosure also provides methods of preparing the pharmaceutical compositions provided herein. The method can comprise combining a buffer solution having a pH from about 5.0 to about 8.0 (*e.g.*, about 5.0 to about 5.5), a stabilizer (*e.g.*, proline), a surfactant (*e.g.*, Polysorbate 80), and a therapeutically effective amount of one or more anti-OX40 antibodies. One or more of the excipients in the pharmaceutical formulations described herein can be combined with therapeutically effective amounts of one or more anti-OX40 antibodies to produce a wide range of pharmaceutical formulations. The buffer, stabilizer, surfactant, and anti-OX40 antibodies have been described in the sections above.

[0119] Generally, a pharmaceutical formulation should be sterilized, which can be achieved by either using sterile reagents in a sterile manufacturing environment or being sterilized following preparation. For example, sterile pharmaceutical formulations can be prepared by incorporating one or more anti-OX40 antibodies in the required amount into a buffer together with other excipients (*e.g.*, stabilizer and surfactants) described herein, before applying a sterilization method, such as microfiltration.

[0120] For illustrative purposes, in some embodiments, the method of preparing the pharmaceutical formulations provided herein comprises a formulation process comprising the following steps.

[0121] 1) Prepare stock solutions of anti-OX40 antibody in 20 mM glutamic acid/histidine buffer at desired pH (*e.g.*, pH 5.3 or 5.4).

[0122] If needed, the drug substance having anti-OX40 antibody at a lower concentration can be concentrated and exchanged into glutamic acid/histidine buffer using TFF system.

[0123] 2) Add proline from stock solution (*e.g.*, 40% (w/w)) at a target concentration of 2.8% (w/v) according to target quantity of anti-OX40 antibody.

[0124] 3) Add polysorbate from stock solution (*e.g.*, 10% (w/w)) at a target concentration of 0.02% (w/v) according to target quantity of anti-OX40 antibody.

[0125] 4) Adjust to the target concentration (*e.g.*, 120-180 mg/mL, or 150 mg/mL) by adding 20 mM glutamic acid/histidine buffer. Filter the liquid composition through polyvinylidene difluoride (PVDF) membrane.

[0126] The proline stock solution 40% (w/w) and polysorbate 80 stock solution 10% (w/w) can be prepared in 20 mM glutamic acid &histidine at desired pH (*e.g.*, pH 5.3 or 5.4). Order of Steps 2) and 3) can be switched.

[0127] In some embodiments, the method of preparing the pharmaceutical compositions provided herein can further comprise a cell culture process and a purification process before the formulation process as described above. The cell culture process can be carried with vial thaw and seed culture expansion in shake flask, cell expansion in an RM bioreactor, cell expansion in an XDR 200 L bioreactor, production culture in a SUB 500 L bioreactor and depth filtration harvest. The purification process can be carried out using affinity chromatography (AC), low pH virus inactivation and neutralization (VIN), intermediate depth filtration (Int. DF), anion exchange (AEX) chromatography, cation exchange (CEX) chromatography, viral filtration (VF) and Ultrafiltration/Diafiltration (UF/DF).

[0128] The stability of the anti-OX40 antibody in the pharmaceutical compositions prepared above can be assessed using methods known in the art, such as those used in the Examples, including, without limitation, size exclusion chromatography, particle counting, anion/cation exchange chromatography, functional assays, such as binding activity.

5.5 Uses

[0129] The pharmaceutical compositions provided herein can be used in medical treatment. In some embodiments, the pharmaceutical compositions provided herein can be used in treating an OX40-associated disease. In some embodiments, the pharmaceutical compositions provided herein can be used in preparation of a medication for treating an OX40-associated disease. In some embodiments, the present disclosure provides a method of treating OX40-associated disease in a subject in need thereof, comprising administering a therapeutically effective amount of the pharmaceutical formulation of the present disclosure to the subject. An “OX40-associated disease”

refers to a disease that is associated with OX40 signaling or can benefit from intervention of the OX40 signaling.

[0130] In some embodiments, the OX40-associated disease is cancer. In certain embodiments, the cancer is selected from the group consisting of breast cancer, melanoma, small-cell lung cancer, kidney cancer, stomach cancer, liver cancer, ovarian cancer, lymphatic leukemia myeloma, prostate cancer, urothelial cancer, head and neck cancer, non-small cell lung cancer, mesothelioma, skin cancer, lymphoma, leukemia, and sarcoma.

[0131] In some embodiments, the OX40-associated disease is an inflammation disease and/or autoimmune disease. In certain embodiments, the inflammation disease and/or autoimmune disease is selected from the group consisting of idiopathic dermatitis, autoimmune uveitis, scleroderma, multiple sclerosis, lupus (*e.g.*, systemic lupus erythematosus), rheumatoid arthritis, asthma (*e.g.*, allergic asthma), chronic obstructive pulmonary disease (COPD), ulcerative colitis, and graft-versus-host disease (GVHD).

[0132] In some embodiments, the OX40-associated disease is alopecia areata (AA). Provided herein are methods of treating AA in a subject in need thereof comprising administering to the subject in need thereof a therapeutically effective amount of the pharmaceutical compositions described herein. Provided herein are uses of the pharmaceutical compositions disclosed herein for treating AA. In some embodiments, provided herein are uses of the pharmaceutical compositions provided herein for the preparation of a medicament for treating AA.

[0133] n some embodiments, the OX40-associated disease is atopic dermatitis (AD). Provided herein are methods of treating AD in a subject in need thereof comprising administering to the subject in need thereof a therapeutically effective amount of the pharmaceutical compositions described herein. Provided herein are uses of the pharmaceutical compositions disclosed herein for treating AD. In some embodiments, provided herein are uses of the pharmaceutical compositions provided herein for the preparation of a medicament for treating AD.

[0134] The pharmaceutical compositions of the present disclosure can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As appreciated by the skilled artisan, the route and/or mode of administration vary depending upon the desired results. Preferred routes of administration include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. More preferred routes of administration are intravenous or subcutaneous. The phrase “parenteral administration” as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous,

intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Alternatively, the pharmaceutical compositions of the present disclosure can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

[0135] In some embodiments, the pharmaceutical compositions of the present disclosure are suitable for subcutaneous administration. In some embodiments, the pharmaceutical compositions of the present disclosure suitable for subcutaneous administration comprises the monoclonal anti-OX40 antibody or fragment thereof at a concentration ranging from 120 to 180 mg/mL. In some embodiments, the pharmaceutical compositions of the present disclosure suitable for subcutaneous administration comprises the monoclonal anti-OX40 antibody or fragment thereof at a concentration ranging from 140 to 160 mg/mL. In some embodiments, the pharmaceutical compositions of the present disclosure suitable for subcutaneous administration comprises the monoclonal anti-OX40 antibody or fragment thereof at about 150 mg/mL.

[0136] Pharmaceutical compositions disclosed herein can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0137] The pharmaceutical formulation of the present disclosure can be administered at a single or multiple doses. As used herein, the term “dose” or “dosage” are interchangeable and indicates an amount of drug substance administered per body weight of a subject or a total dose administered to a subject irrespective of their body weight.

5.6 Kit

[0138] Also provided herein are kits comprising the pharmaceutical compositions, formulations, or dosage forms described herein. In certain embodiments, the kit comprises one or more containers, a

buffer such as glutamic acid &histidine buffer, a stabilizer such as proline, a surfactant such as polysorbate 80, and a monoclonal anti-OX40 antibody or antigen-binding fragment thereof, as well as instructions regarding the use thereof.

[0139] Kits provided herein can be used for the treatment of a disease, disorder or condition, such as an OX40-associated diseases, packaged into suitable packaging material. A kit can include a label or packaging insert including a description of the components or instructions for use *in vitro*, *in vivo*, or *ex vivo*, of the components therein.

[0140] Exemplary instructions include instructions for treatment and/or prevention of a disease or disorder. In some embodiments, the disease or disorder is an OX40-associated diseases. In some embodiments, the disease is an immune or inflammatory disease. In some embodiments, the disease is alopecia areata (AA). In some embodiments, the disease is atopic dermatitis (AD). In some embodiments, the disease is a cancer or tumor.

[0141] The term "packaging material" refers to a physical structure housing the components of the kit. The packaging material can maintain the components sterilely, and can be made of material commonly used for such purposes (*e.g.*, paper, corrugated fiber, glass, plastic, foil, ampules, vials, tubes, etc.).

[0142] Kits provided herein can include labels or inserts. Labels or inserts include "printed matter," *e.g.*, paper or cardboard, separate or affixed to a component, a kit or packing material (*e.g.*, a box), or attached to, for example, an ampule, tube or vial containing a kit component. Labels or inserts can additionally include a computer readable medium, such as a disk (*e.g.*, hard disk, card, memory disk), optical disk such as CD- or DVD-ROM/RAM, DVD, MP3, magnetic tape, or an electrical storage media such as RAM and ROM or hybrids of these such as magnetic/optical storage media, FLASH media or memory type cards. In some embodiments, the instructions recite a method provided herein.

[0143] Labels or inserts can include, among other things, identifying information of one or more components therein, dosing parameters, and/or information on the clinical pharmacology of the active ingredient(s), including mechanism of action, pharmacokinetics and pharmacodynamics. Labels or inserts can include information identifying manufacturer information, lot numbers, manufacturer location and date.

[0144] Labels or inserts can include information on a condition, disorder, disease or symptom for which a kit component may be used. Labels or inserts can include instructions for the clinician or for a subject for using one or more of the kit components in a method, treatment protocol or therapeutic regimen. Instructions can include dosage amounts, frequency or duration, and instructions for practicing any of the methods, treatment protocols or therapeutic regimens set forth herein.

Exemplary instructions include instructions for treatment or use of an anti-OX40 antibody as set forth herein and/or the use of an additional agent or treatment modality useful in treating an OX40-associated disease. Kits provided herein therefore can additionally include labels or instructions for practicing any of the methods and uses provided herein, including treatment methods and uses.

[0145] Labels or inserts can include information on any benefit that a component may provide, such as a prophylactic or therapeutic benefit. Labels or inserts can include information on potential adverse side effects, such as warnings to the subject or clinician regarding situations where it would not be appropriate to use a particular composition. Adverse effects could also occur when the subject has, will be, or is currently taking one or more other medications that may be incompatible with the composition, or the subject has, will be, or is currently undergoing another treatment protocol or therapeutic regimen which would be incompatible with the composition and, therefore, instructions could include information regarding such incompatibilities.

[0146] Kits provided herein can additionally include other components. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package. In certain embodiments, kits are designed for cold storage. Kits provided herein can further be designed to contain the anti-OX40 antibodies provided herein, or that contain nucleic acids encoding the antibodies. Kits provided herein can also be designed to contain, either separately or in combination with the anti-OX40 antibody provided herein, one or more additional agents useful in the treatment or prevention of a disease or disorder provided herein. Any cells in the kit can be maintained under appropriate storage conditions until ready to use.

[0147] In certain embodiments, the kits provided herein comprise one or more single or multi-chambered syringes, such as liquid syringes and lyosyringes, for administering the pharmaceutical formulations of the present disclosure. The kits provided herein can comprise a pharmaceutical formulation for human use.

5.7 Exemplified embodiments

[0148] Embodiment 1: A pharmaceutical composition comprising (A) an anti-OX40 antibody or antigen-binding fragment thereof comprising (1) a light chain variable region (VL) comprising VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:1, 2, and 3, respectively; and (2) a heavy chain variable region (VH) comprising VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of SEQ ID NOs:4, 5 and 6, respectively; and (B) a pharmaceutically acceptable carrier comprising a glutamic/histidine buffer, proline, and polysorbate 80.

[0149] Embodiment 2: The pharmaceutical composition of Embodiment 1, wherein the anti-OX40 antibody or antigen-binding fragment is a chimeric antibody or antigen-binding fragment, a humanized antibody or antigen-binding fragment, or a human antibody or antigen-binding fragment.

[0150] Embodiment 3: The pharmaceutical composition of Embodiment 1, wherein the anti-OX40 antibody or antigen-binding fragment is a humanized antibody or antigen-binding fragment.

[0151] Embodiment 4: The pharmaceutical composition of any one of Embodiments 1 to 3, wherein (1) the VL has at least 85%, at least 90%, at least 95%, at least 98%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:7; and/or (2) the VH has at least 85%, at least 90%, at least 95%, at least 98%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:8.

[0152] Embodiment 5: The pharmaceutical composition of Embodiment 4, wherein the VL has the amino acid sequence of SEQ ID NO:7, 9, or 10, and the VH has the amino acid sequence of SEQ ID NO:8, 11, or 12.

[0153] Embodiment 6: The pharmaceutical composition of Embodiment 4, wherein the VL and VH have the amino acid sequences of SEQ ID NOs:7 and 8, respectively.

[0154] Embodiment 7: The pharmaceutical composition of any one of Embodiments 1 to 6, wherein the anti-OX40 antibody or antigen-binding fragment is selected from the group consisting of a Fab, a Fab', a $F(ab')_2$, a Fv, a scFv, and a $(scFv)_2$.

[0155] Embodiment 8: The pharmaceutical composition of any one of Embodiments 1 to 6, wherein the anti-OX40 antibody or antigen-binding fragment is an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody.

[0156] Embodiment 9: The pharmaceutical composition of Embodiment 8, wherein the anti-OX40 antibody is a humanized IgG1 antibody.

[0157] Embodiment 10: The pharmaceutical composition of Embodiment 9, wherein the anti-OX40 antibody comprises a light chain constant region (CL) having at least 85% sequence identity to kappa CL (Cκ; SEQ ID NO:13) or lambda CL (Cλ; SEQ ID NO:14).

[0158] Embodiment 11: The pharmaceutical composition of Embodiment 10, wherein the CL is Cκ (SEQ ID NO:13).

[0159] Embodiment 12: The pharmaceutical composition of any one of Embodiments 9 to 11, wherein the anti-OX40 antibody comprises the wildtype heavy chain constant region (CH) of human IgG1 (SEQ ID NO:16), or a variant thereof having reduced binding to FcγR.

[0160] Embodiment 13: The pharmaceutical composition of Embodiment 12, wherein the anti-OX40 antibody comprises the CH region of human IgG1 with N297A substitution.

[0161] Embodiment 14: The pharmaceutical composition of Embodiment 9, wherein the anti-OX40 antibody comprises a light chain having the amino acid sequence of SEQ ID NO:19 and a heavy chain having the amino acid sequence of SEQ ID NO:20.

[0162] Embodiment 15: The pharmaceutical composition of any one of Embodiments 1 to 14, comprising the anti-OX40 antibody or antigen-binding fragment at a concentration ranging from 80 to 180 mg/mL, from 100 to 180 mg/mL, from 120 to 180 mg/mL, from 140 to 180 mg/mL, from 100 to 160 mg/mL, or from 120 to 160 mg/mL.

[0163] Embodiment 16: The pharmaceutical composition of Embodiment 15, comprising the antibody or antigen-binding fragment at a concentration ranging from 140 to 160 mg/mL.

[0164] Embodiment 17: The pharmaceutical composition of Embodiment 15, comprising the antibody or antigen-binding fragment at a concentration of about 150 mg/mL.

[0165] Embodiment 18: The pharmaceutical composition of any one of Embodiments 1 to 17, comprising the glutamic/histidine buffer at a concentration ranging from 10 to 50 mM, from 10 to 40 mM, from 10 to 30 mM, or from 15 to 25 mM.

[0166] Embodiment 19: The pharmaceutical composition of Embodiment 18, comprising the glutamic/histidine buffer at 20 mM.

[0167] Embodiment 20: The pharmaceutical composition of any one of Embodiments 1 to 19, comprising the proline at a concentration ranging from 1.0 to 5.0% (w/v), from 1.0 to 4.0% (w/v), from 1.0 to 3.0% (w/v), from 2.0 to 5.0% (w/v), from 2.0 to 4.0% (w/v), or from 2.0 to 3.0% (w/v).

[0168] Embodiment 21: The pharmaceutical composition of Embodiment 20, comprising the proline at a concentration ranging from 2.5 to 3.0% (w/v).

[0169] Embodiment 22: The pharmaceutical composition of Embodiment 20, comprising the proline at 2.8% (w/v).

[0170] Embodiment 23: The pharmaceutical composition of any one of Embodiments 1 to 22, comprising the polysorbate 80 at a concentration ranging from 0.001 to 0.1% (w/v), from 0.005 to 0.05% (/v), or from 0.01 to 0.05% (v/v).

[0171] Embodiment 24: The pharmaceutical composition of Embodiment 23, comprising the polysorbate 80 at a concentration ranging from 0.01 to 0.03% (w/v).

[0172] Embodiment 25: The pharmaceutical composition of Embodiment 23, comprising the polysorbate 80 at 0.02% (w/v).

[0173] Embodiment 26: The pharmaceutical composition of any one of Embodiments 1 to 25, having a pH ranging from 4.8 to 6.8, from 5.0 to 6.5, from 5.0 to 6.0, or from 5.0 to 5.8.

[0174] Embodiment 27: The pharmaceutical composition of Embodiment 26, having a pH ranging from 5.1 to 5.6.

[0175] Embodiment 28: The pharmaceutical composition of Embodiment 26, having a pH of about 5.3 or about 5.4.

[0176] Embodiment 29: The pharmaceutical composition of any one of Embodiments 1 to 17, comprising 20 mM glutamic/histidine buffer, 2.8% (w/v) proline, and 0.01-0.03% (w/v) polysorbate 80, wherein the pH ranges from 5.0 to 5.6.

[0177] Embodiment 30: The pharmaceutical composition of any one of Embodiments 1 to 17, comprising 20 mM glutamic/histidine buffer, 2.8% (w/v) proline, and 0.02% (w/v) polysorbate 80, wherein the pH is about 5.3 or about 5.4.

[0178] Embodiment 31: The pharmaceutical composition of any one of Embodiments 1 to 30, wherein the pharmaceutical composition is in liquid form.

[0179] Embodiment 32: The pharmaceutical composition of any one of Embodiments 1 to 30, wherein the pharmaceutical composition is lyophilized.

[0180] Embodiment 33: The pharmaceutical composition of any one of Embodiments 1 to 32, wherein the viscosity of the pharmaceutical composition is no more than 20 cP, no more than 18 cP, no more than 16 cP, no more than 14 cP, no more than 12 cP, or no more than 10 cP.

[0181] Embodiment 34: The pharmaceutical composition of Embodiment 33, wherein the viscosity is about 12 cP.

[0182] Embodiment 35: The pharmaceutical composition of any one of Embodiments 1 to 34, wherein the osmotic pressure of the pharmaceutical composition ranges from 200 to 400 mOsmol/kg, from 250 to 400 mOsmol/kg, or from 300 to 400 mOsmol/kg.

[0183] Embodiment 36: The pharmaceutical composition of Embodiment 36, wherein the osmotic pressure is about 350 mOsmol/kg.

[0184] Embodiment 37: The pharmaceutical composition of any one of Embodiments 1 to 36, wherein the purity of the antibody or antigen-binding fragment is at least 90%.

[0185] Embodiment 38: The pharmaceutical composition of Embodiment 37, wherein the purity of the antibody or antigen-binding fragment is 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, or at least 99%.

[0186] Embodiment 39: The pharmaceutical composition of Embodiment 37 or 38, wherein the purity of the antibody or antigen-binding fragment is measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC) or Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS).

[0187] Embodiment 40: The pharmaceutical composition of Embodiment 39, wherein the purity of the antibody or antigen-binding fragment is at least 95% measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC).

[0188] Embodiment 41: The pharmaceutical composition of Embodiment 39, wherein the purity of the antibody or antigen-binding fragment is at least 90% measured by Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS).

[0189] Embodiment 42: The pharmaceutical composition of any one of Embodiments 1 to 41, comparing no more than 60% charge variants.

[0190] Embodiment 43: The pharmaceutical composition of Embodiment 42, comprising no more than 55%, no more than 50%, no more than 45%, or no more than 40%, no more than 35%, or no more than 30% charge variants.

[0191] Embodiment 44: The pharmaceutical composition of Embodiment 41 or 43, wherein the charge variants are measured by Cation Exchange-High Performance Liquid Chromatography (CEX-HPLC).

[0192] Embodiment 45: The pharmaceutical composition of any one of Embodiments 1 to 44, wherein the purity of the antibody or antigen-binding fragment is reduced by no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%, or no more than 2% after storage for a period of time that is at least 2 weeks.

[0193] Embodiment 46: The pharmaceutical composition of Embodiment 45, wherein the purity is measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC) or Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS).

[0194] Embodiment 47: The pharmaceutical composition of any one of Embodiments 1 to 46, wherein the charge variants of the antibody or antigen-binding fragment are increased by no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5% after storage for a period of time that is at least 2 weeks.

[0195] Embodiment 48: The pharmaceutical composition of Embodiment 47, wherein the charge variants are measured by Cation Exchange-High Performance Liquid Chromatography (CEX-HPLC).

[0196] Embodiment 49: The pharmaceutical composition of any one of Embodiments 45 to 48, wherein the period of time is at least 2 weeks, at least 4 weeks, at least 2 months, at least 3 months, at least 6 months, at least 9 months, at least 12 months, at least 18 months, or at least 24 months.

[0197] Embodiment 50: The pharmaceutical composition of any one of Embodiments 45 to 49, that is stored at about -80°C, about -20°C, about 4°C, about 25°C or about 40°C.

[0198] Embodiment 51: The pharmaceutical composition of any one of Embodiments 45 to 50, that is stored upright or inverted.

[0199] Embodiment 52: The pharmaceutical composition of any one of Embodiments 45 to 51, that is stored with agitation.

[0200] Embodiment 53: The pharmaceutical composition of any one of Embodiments 1 to 52, wherein the purity of the antibody or antigen-binding fragment is reduced by no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%, or no more than 2% after at least 1, at least 2, at least 3, at least 4, or at least 5 freeze-thaw cycles.

[0201] Embodiment 54: The pharmaceutical composition of Embodiment 53, wherein the purity is measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC) or Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS).

[0202] Embodiment 55: The pharmaceutical composition of any one of Embodiments 1 to 54, wherein the charge variants of the antibody or antigen-binding fragment are increased to no more than 60%, to no more than 55%, no more than 50%, no more than 45%, or no more than 40%, or no more than 35% after at least 1, at least 2, at least 3, at least 4, or at least 5 freeze-thaw cycles.

[0203] Embodiment 56: The pharmaceutical composition of Embodiment 55, wherein the charge variants are measured by Cation Exchange-High Performance Liquid Chromatography (CEX-HPLC).

[0204] Embodiment 57: The pharmaceutical composition of any one of Embodiments 1 to 56 that is suitable for subcutaneous injection.

[0205] Embodiment 58: A method of treating an OX40-associated disease comprising administering a therapeutically effective amount of the pharmaceutical composition of any one of Embodiments 1 to 57.

[0206] Embodiment 59: The method of Embodiment 58 comprising subcutaneous injection.

[0207] Embodiment 60: Use of the pharmaceutical composition of any one of Embodiments 1 to 57 in treating an OX40-associated disease.

[0208] Embodiment 61: Use of the pharmaceutical composition of any one of Embodiments 1 to 57 in preparation of a medication for treating an OX40-associated disease.

[0209] Embodiment 62: The method or use of any one of Embodiments 58 to 61, wherein the OX40-associated disease is alopecia areata (AA) or atopic dermatitis (AD).

[0210] Embodiment 63: A kit comprising the pharmaceutical composition of any one of Embodiments 1 to 57.

5.8 Experimental

[0211] The examples provided below are for purposes of illustration only, which are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teachings provided herein.

[0212] The active pharmaceutical ingredient under investigation of the studies described below is a humanized anti-OX40 IgG1 monoclonal antibody, of which the light chain has the amino acid sequence of SEQ ID NO:19, and the heavy chain has the amino acid sequence of SEQ ID NO:20 (hereinafter the "API").

[0213] Briefly, studies described below development of the high concentrated formulation of the API for subcutaneous injection to be used in clinical trials. This report included solubility profiling study, robustness study and formulation confirmation study.

[0214] Abbreviations: NA: Not applicable; ND: Not detected; NT: Not Tested; RT: Room Temperature; CE-SDS: Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis; SEC-UPLC: Size exclusion- Ultra High Performance Liquid Chromatography; CEX-HPLC: Cation exchange-High Performance Liquid Chromatography; DS: Drug Substance; ELISA: Enzyme-linked immunosorbent assay; HMW: High molecular weight aggregate; LMW: Low molecular weight aggregate; SC: subcutaneous; NOAEL: No Observed Adverse Effect Level.

5.8.1 Example 1: Solubility profiling study

[0215] The solubility profiling study evaluated the solubility, viscosity and stability in buffer system of glutamic acid/histidine at pH 5.3 with different excipients of sucrose and proline at protein concentrations ranging from 80mg/mL to 180mg/mL. A total of 10 groups of formulations were designed and investigated for as long as 4 weeks under conditions of 40±2°C/75±5%RH (upright), 25±2°C/60±5%RH (upright) and 2-8°C (upright).

Table 4: Design of solubility profiling study

No.	Protein concentration (mg/mL)	Formulation (w/v)
F1	80	20mM glutamate/histidine buffer pH 5.3, 7.5% sucrose, 0.02% polysorbate 80
F2	100	

No.	Protein concentration (mg/mL)	Formulation (w/v)
F3	120	
F4	150	
F5	180	
F6	80	20mM glutamate/histidine buffer pH 5.3, 2.8% proline, 0.02% polysorbate 80
F7	100	
F8	120	
F9	150	
F10	180	

[0216] At all timepoints, samples were analyzed for appearance (color, clarity, visible foreign matter), pH, CEX-HPLC, SEC-UPLC, CE-SDS (non-reduced), and protein content. At T0, samples were also measured for viscosity and osmotic pressure. For samples stored at 40±2°C/75%±5% RH (Upright), after 4 weeks of storage, samples were also analyzed for insoluble particle.

[0217] Osmotic pressure and viscosity: The osmotic pressure and viscosity results are shown in the table below. The study results showed that only F5 and F10 exhibited viscosity greater than 20 cP, and the viscosity of proline-containing formulations were significantly lower than that of sucrose-containing formulations as the protein concentration increased. However, the osmolality of all formulations was within the acceptable range.

Table 5: Results of osmotic pressure and viscosity for Solubility profiling study

No.	osmotic pressure mOsmol/kg	Viscosity cP, 25°C
F1	273	3.5
F2	279	5.0
F3	281	7.9
F4	295	15.4
F5	309	34.0
F6	283	2.8
F7	288	4.2
F8	288	6.2

No.	osmotic pressure mOsmol/kg	Viscosity cP, 25°C
F9	296	11.7
F10	306	22.7

[0218] Appearance, pH and protein content: No significant changes in appearance (color, clarity, visible foreign matter), pH or protein content were observed across all tested formulations under respective tested conditions. All samples were observed to be sightly yellow, slightly opalescence, and free of particles.

[0219] SEC-UPLC, CEX-HPLC, CE-SDS (Non-reduced): The SEC-UPLC results indicated that after 4 weeks of storage at 40 ±2°C/75% ±5% RH (upright), protein purity shown by the main peak decreased to a greater extent in F5 and F10 (by about 4% compared to less than 3% in other groups). The amount of HMW aggregates increased by 2.9% and 3.3% in F5 and F10, respectively, also relatively higher than the other groups. After 4 weeks of storage at 25±2°C/60%±5% RH (upright) and at 2-8 °C (upright), no significant change was observed in main peak purity of protein.

[0220] The CEX-HPLC results indicated that after 4 weeks of storage at 40±2°C/75%±5% RH (upright), protein purity as indicated by the main peak decreased by about 20% in all formulations. After 4 weeks of storage at 25±2°C/ 60%±5% RH (upright), the main peak of all formulations decreased by about 2.5%. After 4 weeks of storage at 2-8°C (upright) there was no significant change in main peak purity of protein.

[0221] The CE-SDS (Non-reduced) results indicated that after 4 weeks of storage at 40±2°C/75%±5% RH (upright), protein purity decreased similarly and LMW fragments increased by about 2.5% in all formulations. After 4 weeks of storage at 25±2°C/60%±5%RH (upright) and 2-8 °C (upright), there was no significant changes in the purity of protein.

Table 6: Results of SEC-UPLC, CEX-HPLC and CE-SDS for Solubility profiling study

No.	Condition	Time	SEC-UPLC			CEX-HPLC			CE-SDS (Non-reduced)*	
			Main peak (%)	HMW (%)	LMW (s%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
F1	N/A	T0	99.4	0.6	ND	9.3	14.4	76.3	94.4	5.4
		2 Weeks	99.3	0.7	ND	9.4	14.9	75.7	97.2	2.8

No.	Condition	Time	SEC-UPLC			CEX-HPLC			CE-SDS (Non-reduced)*	
			Main peak (%)	HMW (%)	LMW (s%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
	2-8℃ (Upright)	4 Weeks	99.3	0.7	ND	9.7	14.3	76.0	97.1	2.7
	25±2℃/60% ±5% RH (Upright)	2 Weeks	99.2	0.8	ND	9.9	15.4	74.6	96.8	2.9
		4 Weeks	99.2	0.8	0.1	10.0	16.2	73.8	96.6	2.9
	40±2℃/75% ±5% RH (Upright)	2 Weeks	98.3	1.5	0.2	12.1	23.7	64.2	95.3	3.9
		4 Weeks	97.6	1.9	0.5	14.4	30.6	55.0	94.2	5.0
F2	N/A	T0	99.4	0.6	ND	9.2	14.4	76.3	94.4	5.5
	2-8℃ (Upright)	2 Weeks	99.3	0.7	ND	9.4	14.9	75.6	97.1	2.7
		4 Weeks	99.3	0.7	ND	9.8	14.9	75.2	97.1	2.7
	25±2℃/60% ±5% RH (Upright)	2 Weeks	99.1	0.9	ND	9.8	14.9	75.4	96.8	2.9
		4 Weeks	99.0	0.9	0.1	9.9	15.5	74.6	96.6	2.9
	40±2℃/75% ±5% RH (Upright)	2 Weeks	98.2	1.6	0.2	13.3	23.9	62.8	95.2	3.9
		4 Weeks	97.4	2.1	0.5	14.7	30.4	54.9	94.1	4.9
F3	N/A	T0	99.4	0.6	ND	9.3	14.5	76.2	94.3	5.5
	2-8℃ (Upright)	2 Weeks	99.1	0.9	ND	9.5	14.9	75.7	97.0	2.7
		4 Weeks	99.3	0.7	ND	9.8	14.9	75.3	97.0	2.7
	25±2℃/60% ±5% RH (Upright)	2 Weeks	99.1	0.9	ND	9.9	15.5	74.6	96.7	2.8
		4 Weeks	99.0	1.0	0.1	9.9	16.0	74.0	96.6	2.9
	40±2℃/75% ±5% RH (Upright)	2 Weeks	98.0	1.8	0.2	12.1	22.8	65.1	95.3	3.9
		4 Weeks	97.2	2.4	0.5	14.7	30.3	54.9	94.2	4.8
F4	N/A	T0	99.3	0.7	ND	9.3	14.7	76.0	94.3	5.4
		2 Weeks	99.2	0.8	ND	9.5	14.8	75.7	97.0	2.6

No.	Condition	Time	SEC-UPLC			CEX-HPLC			CE-SDS (Non-reduced)*	
			Main peak (%)	HMW (%)	LMW (s%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
	2-8℃ (Upright)	4 Weeks	99.2	0.8	ND	9.5	14.4	76.1	97.0	2.6
	25±2℃/60% ±5% RH (Upright)	2 Weeks	99.0	1.0	ND	9.6	14.8	75.6	96.8	2.9
		4 Weeks	98.9	1.0	0.1	10.5	16.1	73.4	96.6	2.9
	40±2℃/75% ±5% RH (Upright)	2 Weeks	97.7	2.1	0.2	12.3	23.6	64.1	95.2	3.8
		4 Weeks	96.7	2.7	0.5	13.9	30.2	55.9	94.1	4.9
F5	N/A	T0	99.3	0.7	ND	9.5	14.6	76.0	94.3	5.5
	2-8℃ (Upright)	2 Weeks	99.2	0.8	ND	9.7	14.9	75.5	97.1	2.6
		4 Weeks	99.2	0.8	ND	9.6	14.8	75.5	97.0	2.6
	25±2℃/60% ±5% RH (Upright)	2 Weeks	98.9	1.1	ND	10.1	15.5	74.4	96.5	2.9
		4 Weeks	98.8	1.2	0.1	10.6	16.1	73.2	96.5	2.9
	40±2℃/75% ±5% RH (Upright)	2 Weeks	97.1	2.6	0.2	12.4	23.3	64.4	95.0	3.9
		4 Weeks	95.9	3.6	0.5	14.3	29.9	55.7	93.9	4.8
F6	N/A	T0	99.4	0.6	ND	9.4	14.6	76.0	94.3	5.5
	2-8℃ (Upright)	2 Weeks	99.3	0.7	ND	9.5	15.0	75.4	97.1	2.8
		4 Weeks	99.4	0.6	ND	9.4	14.8	75.8	97.0	2.8
	25±2℃/60% ±5% RH (Upright)	2 Weeks	99.2	0.8	ND	9.9	15.5	74.7	96.7	3.0
		4 Weeks	99.1	0.8	0.1	10.1	16.2	73.8	96.7	3.0
	40±2℃/75% ±5% RH (Upright)	2 Weeks	98.4	1.4	0.2	12.2	22.8	65.0	95.2	4.2
		4 Weeks	97.7	1.9	0.5	14.0	29.9	56.1	94.1	5.3
F7	N/A	T0	99.4	0.6	ND	9.3	14.6	76.1	94.2	5.7
		2 Weeks	99.3	0.7	ND	9.5	14.8	75.7	97.1	2.7

No.	Condition	Time	SEC-UPLC			CEX-HPLC			CE-SDS (Non-reduced)*	
			Main peak (%)	HMW (%)	LMW (s%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
	2-8℃ (Upright)	4 Weeks	99.3	0.7	ND	9.4	14.9	75.7	97.0	2.7
	25±2℃/60% ±5% RH (Upright)	2 Weeks	99.1	0.9	ND	10.0	15.4	74.5	96.8	2.8
		4 Weeks	99.2	0.8	0.1	10.6	16.3	73.1	96.6	3.0
	40±2℃/75% ±5% RH (Upright)	2 Weeks	98.1	1.6	0.2	12.4	23.5	64.1	95.0	4.2
		4 Weeks	97.4	2.1	0.5	15.2	30.1	54.8	94.5	5.5
F8	N/A	T0	99.4	0.6	ND	9.3	14.5	76.1	94.2	5.7
	2-8℃ (Upright)	2 Weeks	99.3	0.7	ND	9.6	14.9	75.5	97.0	2.7
		4 Weeks	99.3	0.7	ND	9.5	14.8	75.7	97.2	2.6
	25±2℃/60% ±5% RH (Upright)	2 Weeks	99.1	0.9	ND	10.0	15.4	74.6	96.7	2.9
		4 Weeks	99.0	0.9	0.1	10.7	16.1	73.2	96.7	3.0
	40±2℃/75% ±5% RH (Upright)	2 Weeks	98.0	1.8	0.2	12.5	23.3	64.2	94.9	4.3
		4 Weeks	97.1	2.4	0.5	15.0	29.0	56.1	94.6	5.4
F9	N/A	T0	99.3	0.7	ND	9.4	14.7	75.9	94.2	5.7
	2-8℃ (Upright)	2 Weeks	99.2	0.8	ND	9.7	14.9	75.5	97.1	2.6
		4 Weeks	99.4	0.6	ND	10.0	14.8	75.2	97.1	2.6
	25±2℃/60% ±5% RH (Upright)	2 Weeks	99.1	0.9	ND	10.0	15.5	74.4	96.6	3.0
		4 Weeks	98.9	1.0	0.1	10.8	16.1	73.1	96.5	3.1
	40±2℃/75% ±5% RH (Upright)	2 Weeks	97.6	2.2	0.2	13.6	23.2	63.2	94.8	4.3
		4 Weeks	96.5	3.0	0.5	15.5	29.7	54.9	94.5	5.4
F10	N/A	T0	99.3	0.7	ND	9.5	13.9	76.6	94.1	5.7
		2 Weeks	99.2	0.8	ND	9.7	14.8	75.5	97.0	2.8

No.	Condition	Time	SEC-UPLC			CEX-HPLC			CE-SDS (Non-reduced)*	
			Main peak (%)	HMW (%)	LMW (s%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
	2-8℃ (Upright)	4 Weeks	99.2	0.8	ND	9.7	14.7	75.7	97.1	2.6
	25±2℃/60% ±5% RH (Upright)	2 Weeks	99.0	1.0	ND	10.2	15.4	74.4	96.6	2.9
		4 Weeks	98.8	1.1	0.1	10.9	16.1	73.0	96.5	3.0
	40±2℃/75% ±5% RH (Upright)	2 Weeks	97.0	2.8	0.2	12.6	22.4	65.0	94.7	4.2
		4 Weeks	95.4	4.0	0.6	14.7	29.3	56.0	94.5	5.4

Note: The purity of CE-SDS (non-reduced) T0 sample appeared abnormally low, likely due to pre-treatment.

[0222] Insoluble particles: As summarized in the table below, after 4 weeks of storage at 40±2℃/75%±5% RH (upright), the levels of insoluble particles remained low in all tested samples. No significant changes were detected.

Table 7: Result of insoluble particles for solubility study

No.	Study condition	Time	MFI number/mL			
			≥2μm, <5μm	≥5μm, < 10μm	≥10μm	≥25μm
F1	N/A	T0	395	81	10	0
	40±2℃/75%± 5% RH (Upright)	4 Weeks	NT	NT	NT	NT
F2	N/A	T0	133	12	0	0
	40±2℃/75%± 5% RH (Upright)	4 Weeks	776	68	0	0
F3	N/A	T0	297	24	0	0
	40±2℃/75%± 5% RH (Upright)	4 Weeks	540	19	0	0
F4	N/A	T0	361	48	0	0

No.	Study condition	Time	MFI number/mL			
			≥2μm, <5μm	≥5μm, < 10μm	≥10μm	≥25μm
	40±2°C/75%± 5% RH (Upright)	4 Weeks	956	87	6	0
F5	N/A	T0	35	6	0	0
	40±2°C/75%± 5% RH (Upright)	4 Weeks	761	162	24	0
F6	N/A	T0	1018	0	0	0
	40±2°C/75%± 5% RH (Upright)	4 Weeks	281	25	6	0
F7	N/A	T0	259	12	0	0
	40±2°C/75%± 5% RH (Upright)	4 Weeks	683	25	0	0
F8	N/A	T0	458	0	6	0
	40±2°C/75%± 5% RH (Upright)	4 Weeks	1302	19	6	0
F9	N/A	T0	120	6	0	0
	40±2°C/75%± 5% RH (Upright)	4 Weeks	957	35	5	0
F10	N/A	T0	120	0	0	0
	40±2°C/75%± 5% RH (Upright)	4 Weeks	829	41	0	0

[0223] Conclusions: The SEC-UPLC data showed some correlation between formation of HMW aggregates and high protein concentration. As the protein concentration increased (up to 180 mg/mL), the formation of HMW aggregates was accelerated. However, no significant difference in the stability of the API was detected in formulations containing proline or sucrose. CEX-HPLC data showed that there were no differences in chemical stability of the API in formulations containing proline or sucrose.

[0224] Viscosity data showed that proline significantly reduced viscosity compared to sucrose, especially in formulations with higher protein concentrations.

[0225] Therefore, formulations having 150 mg/mL protein, 20 mM glutamate/histidine buffer, 2.8% (w/v) proline, and 0.02% polysorbate 80 (w/v) were further studied for robustness.

5.8.2 Example 2: Robustness study

[0226] Robustness study was carried out to evaluate the stability of the formulations having protein concentrations of 140-160 mg/mL at pH of 4.7-5.6 and having polysorbate 80 from 0.01-0.03% (w/v). A total of 8 groups of formulations were investigated for stability after storage for 4 weeks at 40±2°C/75±5%RH (upright), for 3 months at 25±2°C/60±5%RH (upright) and at 2-8 °C (upright), 5 cycles of freezing-thawing (from -70°C to RT, upright), and 5 days of shaking (200 rpm, 25°C, protected from light, upright). The final recommended range of protein concentration was 140 mg/mL - 160 mg/mL (target concentration at 150 mg/mL) with polysorbate 80 concentrations ranging from 0.01% (w/v) - 0.03% (w/v) (target concentration at 0.02% (w/v)) and buffer system of glutamate/histidine with pH ranging from 5.1 to 5.6 (target pH at 5.3).

Table 8: Formulations of Robustness Study

No.	Protein (mg/ml)	Formulation (w/v)
F1	160	20 mM glutamate/histidine buffer, pH 4.7, 2.8% proline, 0.02% polysorbate 80
F2	140	20 mM glutamate/histidine buffer, pH 4.7, 2.8% proline, 0.02% polysorbate 80
F3	160	20 mM glutamate/histidine buffer, pH 5.0, 2.8% proline, 0.02% polysorbate 80
F4	140	20 mM glutamate/histidine buffer, pH 5.0, 2.8% proline, 0.02% polysorbate 80
F5	160	20 mM glutamate/histidine buffer, pH 5.6, 2.8% proline, 0.02% polysorbate 80
F6	140	20 mM glutamate/histidine buffer, pH 5.6, 2.8% proline, 0.02% polysorbate 80
F7	150	20 mM glutamate/histidine buffer, pH 5.3, 2.8% proline, 0.01% polysorbate 80
F8	150	20 mM glutamate/histidine buffer, pH 5.3, 2.8% proline, 0.03% polysorbate 80

Table 9: Protocol of Robustness Study

Study condition		T0	Sampling timepoints and analysis			
Long-term	2-8°C (Upright)	X, Y, W	2 Weeks	4 Weeks	2 months	3 months
			X	X, Y	NT	X, Y
Accelerated	25±2°C/60%±5% RH (Upright)		X	X, Y	X	X, Y

High temperature	40±2°C/75%±5%RH (Upright)		X	X, Y, Z	NT	NT
Freeze-Thaw	-70°C to RT		3 rounds		5 rounds	
			X		X, Y	
Shaking	200 rpm, protected from light, 25°C (upright)		3 days		5 days	
			X		X, Y	

[0227] X: Appearance (color, clarity, visible particles), pH, CEX-HPLC, SEC-UPLC, CE-SDS (non-reduced), Protein content; Y: Insoluble particles; Z: Biological activity (only representative formulation were tested); W: Osmolality and viscosity (only representative formulation were tested)

[0228] Osmolality, viscosity and polysorbate 80 content: The viscosity of F1 and F5 were acceptable (<20 cP). The osmolality of all formulations was acceptable and the polysorbate 80 content of all formulations met the target value.

Table 10: Results of osmolality and viscosity for robustness study

No.	Osmolality (mOsmol/kg)	Viscosity (cP), 25°C	Polysorbate 80 (w/v)
F1	360	15.6	0.023%
F2	347	NT	0.023%
F3	355	NT	0.023%
F4	340	NT	0.023%
F5	344	16.7	0.022%
F6	333	NT	0.022%
F7	349	NT	0.014%
F8	343	NT	0.032%

[0229] Appearance, pH and protein content: No significant changes in appearance (color, clarity, visible foreign matter), pH or protein content were observed across all tested formulations under respective tested conditions. All samples were observed to be sightly yellow, slightly opalescence, and free of particles.

[0230] SEC-UPLC, CEX-HPLC, CE-SDS (Non-reduced): The SEC-UPLC results showed that after storage for 4 weeks at 40±2°C/75%±5%RH (upright), the main peak purity in F1 and F2 (pH 4.7) decreased by 21.0% and 15.3%, and the HMW aggregates increased by 20.2% and 14.6%. By comparison, the main peak in F3 and F4 (pH 5.0) decreased by 5.9% and 4.3% and the HMW increased by 5.4% and 3.9%. The HMW in the remaining formulations increased by only about

2.5%. After storage for 3 months at 25±2 °C/60%±5% RH (upright), the main peak in F1 and F2 (pH 4.7) decreased by 3.6% and 3.3%, and HMW increased by 1.6% and 1.3%, whereas the rest of the formulations showed about 2.5% decreases in main peak and about 1.0% increases in HMW. After storage for 3 months at 2-8°C (upright), no significant changes in the main peak were observed for all formulations. After 5 rounds of freeze-thawing (-70°C to RT) and 5 days of shaking (200 rpm, protected from light, 25°C (upright)), no significant changes were observed in the main peak for all formulations.

[0231] The CEX-HPLC results showed that after storage for 4 weeks at 40±2°C/75%±5% RH (upright), there was a decrease in main peak by approximately 20% for all formulations. After storage for 3 months at 25±2°C/60%±5% RH (upright), the main peak decreased by approximately 7% for all formulations. After storage for 3 months at 2-8°C (upright), no significant changes in the main peak were observed for all formulations. After 5 rounds of freeze-thawing (-70°C to RT) and 5 days of shaking (200 rpm, protected from light, 25°C, upright), no significant changes in the main protein peak were observed for all formulations.

[0232] The results of CE-SDS (non-reduced) showed that after storage for 4 weeks at 40±2°C/75%±5% RH (upright), the protein purity of F1 and F2 (pH 4.7) decreased by 6.2% and 5.8%, respectively, with an increase of about 4.4% in the LMW in both, while the rest of the formulations showed about 3.8% decrease in protein purity and about 2.8% increase in LMW. After storage for 3 months at 25±2°C/60%±5% RH (upright), F1 and F2 (pH 4.7) showed a decrease in protein purity by 2.9% and 2.8% and an increase in LMW content by 1.8% and 1.9%, respectively. The rest of formulations showed less decrease in protein purity by about 2.0% and less increase in LMW content by about 1.3%. After storage for 3 months at 2-8°C (upright), no significant changes in protein purity were observed for all formulations. After 5 rounds of freeze-thawing (-70°C to RT) and 5 days of shaking (200 rpm, protected from light, 25°C, upright), no significant changes in protein purity were observed for all formulations.

Table 11: Results of SEC-UPLC, CEX-HPLC and CE-SDS for Solubility profiling study

No.	Study conditions	Sampling timepoint	SEC-UPLC			CEX-HPLC			CE-SDS (non-reduced)	
			Main peak (%)	HMW (%)	LMW (%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
F1	N/A	T0	99.5	0.5	ND	9.5	14.0	76.5	97.1	2.6

No.	Study conditions	Sampling timepoint	SEC-UPLC			CEX-HPLC			CE-SDS (non-reduced)	
			Main peak (%)	HMW (%)	LMW (%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
	2-8℃ (upright)	2 weeks	99.4	0.6	ND	9.7	14.1	76.2	97.2	2.6
		4 weeks	99.3	0.7	ND	10.3	13.8	75.9	97.1	2.6
		3 months	99.0	1.0	ND	9.8	13.3	76.9	96.8	2.8
	25±2℃/60%±5% RH (upright)	2 weeks	99.2	0.8	ND	10.8	14.3	74.9	96.7	2.8
		4 weeks	98.9	1.0	0.1	12.2	15.2	72.6	96.1	3.2
		2 months	98.0	1.8	0.2	13.0	17.4	69.6	95.0	4.0
		3 months	95.9	2.1	2.0	10.1	19.6	70.3	94.3	4.5
	40±2℃/75% ±5% RH (upright)	2 weeks	86.8	12.5	0.6	12.7	19.7	67.6	93.5	5.0
		4 weeks	78.5	20.7	0.9	20.0	26.1	53.9	90.9	7.0
	Freeze-thaw (-70°C to RT)	3 rounds	99.4	0.6	ND	9.1	14.0	76.9	97.1	2.7
		5 rounds	99.3	0.7	ND	9.1	14.1	76.8	97.2	2.7
	Shaking (200 rpm, protected from light, 25°C (upright))	3 days	99.2	0.8	ND	9.4	14.2	76.4	97.0	2.7
		5 days	99.2	0.8	ND	9.2	13.8	77.0	97.0	2.7
F2	N/A	T0	99.4	0.6	ND	9.5	14.0	76.5	97.1	2.7
	2-8℃ (upright)	2 weeks	99.4	0.6	ND	9.7	13.8	76.5	97.2	2.6
		4 weeks	99.3	0.7	ND	10.3	14.2	75.5	97.1	2.6
		3 months	99.1	0.9	ND	9.7	13.2	77.0	97.0	2.7
	25±2℃/60%±5% RH (upright)	2 weeks	99.2	0.8	ND	10.7	14.6	74.7	96.8	2.8
		4 weeks	99.0	0.9	0.0	12.2	15.3	72.5	96.1	3.2
		2 months	98.2	1.6	0.2	13.0	15.5	71.5	95.0	4.0
		3 months	96.1	1.9	2.1	10.6	19.9	69.6	94.3	4.6
	40±2℃/75% ±5% RH (upright)	2 weeks	90.8	8.6	0.6	13.9	20.1	66.0	93.9	4.9
		4 weeks	84.1	15.2	0.7	20.2	26.7	53.1	91.3	7.1
	Freeze-thaw (-70°C to RT)	3 rounds	99.4	0.6	ND	9.1	14.0	76.9	97.1	2.7
		5 rounds	99.3	0.7	ND	9.1	14.1	76.8	97.2	2.7

No.	Study conditions	Sampling timepoint	SEC-UPLC			CEX-HPLC			CE-SDS (non-reduced)	
			Main peak (%)	HMW (%)	LMW (%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
	Shaking (200 rpm, protected from light, 25°C (upright))	3 days	99.3	0.7	ND	9.3	13.7	77.0	97.0	2.7
		5 days	99.4	0.6	ND	9.1	14.1	76.7	97.0	2.7
F3	N/A	T0	99.4	0.6	ND	9.3	13.7	77.0	97.2	2.6
	2-8°C (upright)	2 weeks	99.4	0.6	ND	9.4	13.9	76.7	97.2	2.6
		4 weeks	99.3	0.7	ND	10.1	14.3	75.6	97.2	2.6
		3 months	99.0	1.0	ND	9.5	13.5	77.0	96.8	2.8
	25±2°C/60%±5% RH (upright)	2 weeks	99.2	0.8	ND	10.2	14.5	75.4	96.8	2.7
		4 weeks	98.9	1.1	ND	11.2	15.4	73.4	96.4	3.0
		2 months	98.3	1.6	0.1	11.3	17.8	70.9	95.3	3.8
		3 months	96.7	1.8	1.5	10.3	19.7	69.9	94.8	4.2
	40±2°C/75% ±5% RH (upright)	2 weeks	96.0	3.5	0.5	12.2	22.0	65.8	94.6	4.2
		4 weeks	93.5	6.0	0.4	15.5	29.7	54.8	92.9	5.8
	Freeze-thaw (-70°C to RT)	3 rounds	99.4	0.6	ND	8.9	14.1	76.9	97.1	2.7
		5 rounds	99.3	0.7	ND	8.9	13.7	77.5	97.2	2.7
	Shaking (200 rpm, protected from light, 25°C (upright))	3 days	99.3	0.7	ND	9.1	14.3	76.6	97.0	2.7
		5 days	99.2	0.8	ND	8.9	13.9	77.2	97.0	2.7
F4	N/A	T0	99.4	0.6	ND	9.4	14.1	76.5	97.2	2.6
	2-8°C (upright)	2 weeks	99.3	0.7	ND	9.5	14.4	76.2	97.2	2.5
		4 weeks	99.3	0.7	ND	9.8	14.3	75.9	97.1	2.6
		3 months	98.9	1.1	ND	9.5	13.8	76.7	96.8	2.7
	25±2°C/60%±5% RH (upright)	2 weeks	99.3	0.7	ND	10.2	14.5	75.3	96.8	2.7
		4 weeks	99.0	1.0	ND	11.3	15.8	72.9	96.5	2.9
		2 months	98.4	1.5	0.1	11.3	17.9	70.8	95.4	3.8
		3 months	96.8	1.6	1.5	10.8	20.0	69.2	95.1	4.0

No.	Study conditions	Sampling timepoint	SEC-UPLC			CEX-HPLC			CE-SDS (non-reduced)	
			Main peak (%)	HMW (%)	LMW (%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
	40±2°C/75% ±5% RH (upright)	2 weeks	96.7	2.8	0.5	11.6	22.1	66.3	94.8	4.2
		4 weeks	95.1	4.5	0.4	15.5	30.2	54.3	93.1	5.7
	Freeze-thaw (-70°C to RT)	3 rounds	99.4	0.6	ND	9.0	14.1	76.9	97.1	2.6
		5 rounds	99.3	0.7	ND	8.9	13.7	77.4	97.2	2.6
	Shaking (200 rpm, protected from light, 25°C (upright))	3 days	99.3	0.7	ND	9.1	14.3	76.6	97.0	2.7
		5 days	99.2	0.8	ND	8.9	14.3	76.8	97.0	2.7
F5	N/A	T0	99.3	0.7	ND	9.1	14.3	76.6	97.1	2.6
	2-8°C (upright)	2 weeks	99.2	0.8	ND	9.2	14.4	76.4	97.1	2.5
		4 weeks	99.2	0.8	ND	9.6	14.2	76.2	97.0	2.6
		3 months	98.9	1.1	ND	9.1	13.8	77.1	96.8	2.7
	25±2°C/60%±5% RH (upright)	2 weeks	99.2	0.8	ND	9.9	15.1	75.0	96.8	2.7
		4 weeks	98.9	1.1	ND	10.4	16.2	73.4	96.4	3.0
		2 months	98.4	1.5	0.1	10.1	18.7	71.2	95.6	3.6
		3 months	97.1	1.6	1.2	9.8	20.0	70.1	95.2	3.7
	40±2°C/75% ±5% RH (upright)	2 weeks	97.1	2.5	0.5	11.9	22.9	65.2	94.8	4.0
		4 weeks	96.6	3.1	0.3	12.6	31.6	55.8	93.5	5.4
	Freeze-thaw (-70°C to RT)	3 rounds	99.3	0.7	ND	8.7	13.8	77.5	97.1	2.6
		5 rounds	99.2	0.8	ND	8.8	14.5	76.7	97.2	2.7
	Shaking (200 rpm, protected from light, 25°C (upright))	3 days	99.3	0.7	ND	8.7	14.5	76.8	97.0	2.7
		5 days	99.1	0.9	ND	8.6	14.6	76.7	97.0	2.6
F6	N/A	T0	99.4	0.6	ND	9.2	14.3	76.5	97.0	2.7
	2-8°C (upright)	2 weeks	99.3	0.7	ND	9.3	14.5	76.2	97.1	2.6
		4 weeks	99.3	0.7	ND	9.4	14.1	76.5	97.0	2.6
		3 months	99.0	1.0	ND	9.1	13.8	77.1	96.9	2.7

No.	Study conditions	Sampling timepoint	SEC-UPLC			CEX-HPLC			CE-SDS (non-reduced)	
			Main peak (%)	HMW (%)	LMW (%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
	25±2°C/60%±5% RH (upright)	2 weeks	99.1	0.9	ND	9.6	14.8	75.6	96.8	2.7
		4 weeks	99.0	1.0	ND	10.5	16.3	73.2	96.3	3.0
		2 months	98.5	1.4	0.1	10.1	18.8	71.1	95.8	3.4
		3 months	97.2	1.5	1.3	9.8	20.2	70.0	95.2	3.8
	40±2°C/75% ±5% RH (upright)	2 weeks	97.3	2.2	0.5	11.8	22.9	65.3	94.9	4.1
		4 weeks	97.0	2.6	0.3	12.4	31.8	55.7	93.5	5.5
	Freeze-thaw (-70°C to RT)	3 rounds	99.2	0.8	ND	8.7	13.8	77.5	97.1	2.6
		5 rounds	99.3	0.7	ND	8.7	14.4	76.8	97.2	2.7
	Shaking (200 rpm, protected from light, 25°C (upright))	3 days	99.2	0.8	ND	8.7	14.6	76.7	97.0	2.6
		5 days	99.2	0.8	ND	8.5	14.6	76.9	97.0	2.7
F7	N/A	T0	99.5	0.5	ND	9.3	14.2	76.4	97.2	2.6
	2-8°C (upright)	2 weeks	99.4	0.6	ND	9.7	14.1	76.2	97.1	2.6
		4 weeks	99.3	0.7	ND	9.7	14.0	76.3	97.1	2.6
		3 months	99.0	1.0	ND	9.3	13.6	77.1	96.8	2.7
	25±2°C/60%±5% RH (upright)	2 weeks	99.1	0.9	ND	10.1	14.4	75.5	96.9	2.7
		4 weeks	99.0	1.0	ND	10.8	15.5	73.7	96.6	2.9
		2 months	98.5	1.4	0.1	10.6	17.8	71.6	95.8	3.5
		3 months	97.1	1.5	1.4	10.1	19.5	70.3	95.3	3.8
	40±2°C/75% ±5% RH (upright)	2 weeks	97.2	2.3	0.5	12.4	22.5	65.1	95.0	4.1
		4 weeks	96.4	3.2	0.3	14.1	31.2	54.7	93.5	5.4
	Freeze-thaw (-70°C to RT)	3 rounds	99.3	0.7	ND	8.8	14.2	77.0	97.1	2.6
		5 rounds	99.3	0.7	ND	9.0	14.3	76.8	97.3	2.7
	Shaking (200 rpm, protected from light, 25°C (upright))	3 days	99.2	0.8	ND	8.9	13.9	77.2	97.0	2.7
		5 days	99.2	0.8	ND	8.7	14.4	76.8	97.0	2.6

No.	Study conditions	Sampling timepoint	SEC-UPLC			CEX-HPLC			CE-SDS (non-reduced)	
			Main peak (%)	HMW (%)	LMW (%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
F8	N/A	T0	99.4	0.6	ND	9.3	13.8	76.9	97.1	2.6
	2-8°C (upright)	2 weeks	99.3	0.7	ND	9.7	14.4	76.0	97.1	2.6
		4 weeks	99.3	0.7	ND	10.0	14.4	75.6	97.1	2.6
		3 months	98.9	1.1	ND	9.4	13.7	76.9	96.9	2.7
	25±2°C/60%±5% RH (upright)	2 weeks	99.1	0.9	ND	10.2	15.0	74.8	96.7	2.8
		4 weeks	98.9	1.1	ND	10.9	16.0	73.1	96.5	2.9
		2 months	98.5	1.4	0.1	10.7	18.3	71.0	95.6	3.6
		3 months	96.9	1.7	1.4	10.4	20.2	69.5	94.7	4.0
	40±2°C/75% ±5% RH (upright)	2 weeks	97.1	2.5	0.5	12.5	22.6	64.9	94.9	4.1
		4 weeks	96.1	3.6	0.3	13.9	31.2	54.9	93.3	5.8
	Freeze-thaw (-70°C to RT)	3 rounds	99.3	0.7	ND	8.5	13.8	77.7	97.3	2.5
		5 rounds	99.3	0.7	ND	8.9	14.3	76.8	97.3	2.7
	Shaking (200 rpm, protected from light, 25°C (upright))	3 days	99.2	0.8	ND	8.8	14.3	76.9	96.9	2.8
		5 days	99.2	0.8	ND	8.7	14.4	76.9	97.0	2.7

[0233] Insoluble particles: As shown below, after storage for 4 weeks at 40±2°C/75%±5% RH (upright), for 3 months at 25±2°C/60%±5% RH (upright), and for 3-month study at 2-8°C (upright), insoluble particles (< 25μm) increased by a small amount but remained at low levels in all formulations. Under tested conditions of 5 rounds of freezing and thawing (-70°C to RT) and 5 days of shaking (200 rpm, protected from light, 25°C, upright), the amount of insoluble particles were not shown significant changes in all formulations, and maintained at low levels.

Table 12: Results of insoluble particles for robustness study

No.	Study condition	Sampling timepoint	MFI number/mL			
			≥2μm, <5μm	≥5μm, < 10μm	≥10μm	≥25μm
F1	N/A	T0	296	48	7	0

No.	Study condition	Sampling timepoint	MFI number/mL			
			≥2μm, <5μm	≥5μm, < 10μm	≥10μm	≥25μm
	2-8°C (upright)	3 months	99	12	4	0
	25±2°C/60%±5% RH (upright)	4 weeks	527	42	0	0
		3 months	525	28	7	0
	40±2°C/75%±5% RH (upright)	4 weeks	759	145	36	0
	Freeze-thaw (-70°C to RT)	5 rounds	362	28	0	0
	Shaking (200 rpm, protected from light, 25°C (upright))	5 days	402	119	0	0
F2	N/A	T0	306	9	0	0
	2-8°C (upright)	3 months	762	60	26	4
	25±2°C/60%±5% RH (upright)	4 weeks	1012	76	13	0
		3 months	277	16	0	0
	40±2°C/75%±5% RH (upright)	4 weeks	434	193	48	0
	Freeze-thaw (-70°C to RT)	5 rounds	388	28	0	0
	Shaking (200 rpm, protected from light, 25°C (upright))	5 days	213	36	0	0
F3	N/A	T0	63	9	0	0
	2-8°C (upright)	3 months	569	49	13	0
	25±2°C/60%±5% RH (upright)	4 weeks	729	108	0	0
		3 months	833	42	7	0
	40±2°C/75%±5% RH (upright)	4 weeks	928	253	0	0
	Freeze-thaw (-70°C to RT)	5 rounds	614	50	0	0
	Shaking (200 rpm, protected from light, 25°C (upright))	5 days	348	16	0	0
F4	N/A	T0	443	27	0	0
	2-8°C (upright)	3 months	488	42	0	0
		4 weeks	607	43	29	0

No.	Study condition	Sampling timepoint	MFI number/mL			
			≥2μm, <5μm	≥5μm, < 10μm	≥10μm	≥25μm
	25±2°C/60%±5% RH (upright)	3 months	101	7	10	0
	40±2°C/75%±5% RH (upright)	4 weeks	1813	42	42	0
	Freeze-thaw (-70°C to RT)	5 rounds	434	13	0	0
	Shaking (200 rpm, protected from light, 25°C (upright))	5 days	143	14	0	0
F5	N/A	T0	316	9	0	0
	2-8°C (upright)	3 months	1319	194	36	0
	25±2°C/60%±5% RH (upright)	4 weeks	834	53	0	0
		3 months	4360	335	42	0
	40±2°C/75%±5% RH (upright)	4 weeks	915	234	0	0
	Freeze-thaw (-70°C to RT)	5 rounds	785	19	0	0
	Shaking (200 rpm, protected from light, 25°C (upright))	5 days	261	16	0	0
F6	N/A	T0	190	0	0	0
	2-8°C (upright)	3 months	166	16	16	7
	25±2°C/60%±5% RH (upright)	4 weeks	1195	84	0	0
		3 months	866	23	23	0
	40±2°C/75%±5% RH (upright)	4 weeks	1912	375	0	0
	Freeze-thaw (-70°C 至 RT)	5 rounds	886	19	6	0
	Shaking (200 rpm, protected from light, 25°C (upright))	5 days	262	9	0	0
F7	N/A	T0	190	18	0	0
	2-8°C (upright)	3 months	530	16	0	0
	25±2°C/60%±5% RH (upright)	4 weeks	604	14	0	0
		3 months	134	3	3	0

No.	Study condition	Sampling timepoint	MFI number/mL			
			≥2μm, <5μm	≥5μm, < 10μm	≥10μm	≥25μm
	40±2°C/75%±5% RH (upright)	4 weeks	1341	152	76	0
	Freeze-thaw (-70°C to RT)	5 rounds	900	56	8	0
	Shaking (200 rpm, protected from light, 25°C (upright))	5 days	701	16	0	0
F8	N/A	T0	296	36	0	0
	2-8°C (upright)	3 months	497	25	11	0
	25±2°C/60%±5% RH (upright)	4 weeks	870	61	20	0
		3 months	199	20	10	3
	40±2°C/75%±5% RH (upright)	4 weeks	1527	129	18	0
	Freeze-thaw (-70°C to RT)	5 rounds	1020	65	0	0
	Shaking (200 rpm, protected from light, 25°C (upright))	5 days	193	0	0	0

[0234] Biological activity: Biological activity results are shown in Table 13. After a 4-week study at 40±2°C/75%±5% RH (upright), no significant changes of biological activity were observed for exemplified formulations F1, F3 or F5.

Table 13: Results of biological activity for robustness study

No.	Sampling point	Biological activity
F1	40±2°C/75%±5% RH (upright) for 4 weeks	89%
F3		103%
F5		110%

[0235] Conclusions: SEC-UPLC data showed that at 40±2°C/75%±5% RH (upright), 1) the increase in HMP was accelerated as the protein concentration increased; 2) the HMW increased significantly faster in formulations at pH 4.7 than in formulations having higher pH values; and 3) the HMW increase in formulations having pH 5.0 was acceptable.

[0236] CEX-HPLC data showed no apparent correlation between the protein concentration and optimal pH, even when tested at 40±2°C/75%±5% RH (upright). Also, the chemical stability of the

API appeared essentially the same across all tested formulations having pH values of 4.7-5.6 and polysorbate 80 content of 0.01-0.03% (w/v).

[0237] CE-SDS (non-reducing) data also showed that LMW fragments formation was faster in formulation having pH 4.7 compared to other formulations having higher pH conditions when tested under 40±2°C/75%±5% RH (upright). The rate of LMW formation did not appear to depend on the protein concentration under same pH condition.

[0238] Therefore, the following formulations were selected for further studies: 140-160 mg/mL API, 20 mM glutamate/histidine buffer system, pH 5.1-5.6, and 0.01-0.03% (w/v) polysorbate 80 (w/v). In the confirmation study, the following formulation was selected: 150 mg/mL API, 20 mM glutamate/histidine buffer pH 5.3, 2.8% (w/v) proline, 0.02% (w/v) polysorbate 80.

5.8.3 Example 3: Formulation confirmation study

[0239] Formulation confirmation study was conducted for the formulation (protein concentration of 150 mg/mL API, buffer system of 20 mM glutamate/histidine at pH 5.3, 2.8% (w/v) proline, 0.02% (w/v) polysorbate 80) to investigate stability for as long as 4 weeks at 40±2°C/75±5%RH (upright), 3 months at 25±2°C/60±5%RH (upright and inverted) and at 2-8°C (upright/inverted), 5 cycles of freezing-thawing (-70°C to RT, upright), and 5 days of shaking (200 rpm, 25°C, protected from light, upright). Data indicated that the recommended formulation supported the storage, shipping and clinical use of the API.

Table 14: Formulation information of confirmation study

No.	Formulation information (w/v)
F1	150 mg/mL protein, 20 mM glutamate/histidine buffer, pH 5.3, 2.8% proline, 0.02% polysorbate 80

Table 15: Study Protocol of formulation confirmation

<table>
<tr><th colspan="2">Study condition</th><th>T0</th><th colspan="9">Sampling timepoint and analysis</th></tr>
<tr><td rowspan="3">Long-term</td><td rowspan="2">2-8°C (upright)</td><td rowspan="4">X, Y, W</td><td>2 weeks</td><td>4 weeks</td><td>2 months</td><td>3 months</td><td>6 months</td><td>9 months</td><td>12 months</td><td>18 months</td><td>24 months</td></tr>
<tr><td>NT</td><td>X, Y</td><td>NT</td><td colspan="6">X, Y</td></tr>
<tr><td>2-8°C (inverted)</td><td colspan="3">NT</td><td>X, Y</td><td colspan="5">NT</td></tr>
<tr><td>Accelerated</td><td>25±2°C/60%±5% RH (upright)</td><td>X</td><td>X, Y</td><td>X</td><td colspan="2">X, Y</td><td colspan="4">NT</td></tr>
</table>

	25±2°C/60%±5% RH (inverted)		NT		X, Y	NT	
High Temperature	40 ±2°C/75%±5%RH (upright)		X	X, Y, Z	NT		
Freeze-Thaw	-70°C to RT		3 rounds			5 rounds	
			X			X，Y	
Shake	200 rpm, protected from light, 25°C (upright)		3 days			5 days	
			X			X，Y	

X=Appearance (color, clarity, visible particles), pH, CEX-HPLC, SEC-UPLC, CE-SDS (non-reduced), Protein content; Y= Insoluble particles; Z= Biological activity; W=Osmolality and viscosity (only test representative formula)

[0240] Osmolality, viscosity and polysorbate 80: The viscosity of F1 was acceptable (<20 cP). The osmolality of F1 was acceptable and the polysorbate 80 content of F1 met the target value.

Table 16: Result of osmolality, viscosity for formulation confirmation study

No.	Osmolality (mOsmol/kg)	Viscosity (cp), 25°C	polysorbate 80 (w/v)
F1	347	11.7	0.024%

Note: Viscosity results were from solubility study.

[0241] Appearance, pH and protein concentration: No significant changes in appearance (color, clarity, visible foreign matter), pH or protein content were observed in the formulations at all time points and under all tested conditions. All samples were observed to be sightly yellow, slightly opalescence, and free of particles.

[0242] SEC-UPLC, CEX-HPLC, CE-SDS (Non-reduced): The SEC-UPLC results showed that the main peak decreased by 3.2% and the HMW increased by 2.9% after a storage for 4 weeks at 40±2°C/75%±5%RH (upright). After storage for 3 months at 25±2°C/60%±5% RH (upright) and 25±2°C/60%±5% RH (inverted), the main peak decreased by 2.4% and 2.3%, respectively, and the HMW increased by 1.0%. After storage for 3 months at 2-8°C (upright) and 2-8°C (inverted), no significant changes in the main peak were observed. After 5 rounds of freeze-thawing (-70°C to RT) and 5 days of shaking (200 rpm, protected from light, 25°C, upright), no significant changes were observed in the main peak.

[0243] The CEX-HPLC results showed a 22.3% decrease in main peak after storage for 4 weeks at 40±2°C/75%±5% RH (upright). After storage for 3 months at 25±2°C/60%±5% RH (upright) and 25±2°C/60%±5% RH (inverted), the main peak decreased by 7.5%. After storage for 3 months at 2-8°C (upright) and 2-8°C (inverted), no significant changes in the main peak were observed. After 5 rounds of freeze-thawing (-70°C to RT) and 5 days of shaking (200 rpm, protected from light, 25°C, upright), no significant changes were observed in the main peak.

[0244] The results of CE-SDS (non-reduced) showed that after storage for 4 weeks at 40±2°C/75%±5% RH (upright), the protein purity decreased by 2.7% and the total LMW increased by 3.0%. After storage for 3 months at 25±2°C/60%±5% RH (upright) and 25±2°C/60%±5% RH (inverted), protein purity decreased by approximately 2.0% and total LMW increased by approximately 1.2%. After storage for 3 months at 2-8°C (upright) and 2-8°C (inverted), no significant changes in protein purity were observed. After 5 rounds of freeze-thawing (-70°C to RT) and 5 days of shaking (200 rpm, protected from light, 25°C, upright), no significant changes in protein purity were observed.

Table 17: Results of SEC-UPLC，CEX-HPLC and CE-SDS (non-reduced) for formulation confirmation study

No.	Study condition	Sampling timepoint	SEC-UPLC			CEX-HPLC			CE-SDS (non-reduced)	
			Main peak (%)	HMW (%)	LMW (%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
F1	N/A	T0	99.3	0.7	ND	9.3	13.8	76.9	97.1	2.6
	2-8°C (upright)	4 weeks	99.3	0.7	ND	10.0	14.5	75.5	97.1	2.5
		3 months	98.9	1.1	ND	9.3	13.5	77.2	97.0	2.7
	2-8°C (inverted)	3 months	98.9	1.1	ND	9.2	13.6	77.2	97.0	2.7
	25±2°C/60%±5% RH (upright)	2 weeks	99.0	1.0	ND	9.4	12.3	78.3	96.8	2.7
		4 weeks	98.9	1.1	ND	10.9	15.7	73.4	96.6	2.9
		2 months	98.4	1.5	0.1	10.7	18.2	71.1	95.5	3.7
		3 months	96.9	1.7	1.4	10.3	19.9	69.8	95.2	3.8
	25±2°C/60%±5% RH (inverted)	3 months	97.0	1.7	1.4	10.5	20.5	69.0	95.1	3.9

No.	Study condition	Sampling timepoint	SEC-UPLC			CEX-HPLC			CE-SDS (non-reduced)	
			Main peak (%)	HMW (%)	LMW (%)	Basic peak (%)	Acid peak (%)	Main peak (%)	Purity (%)	Total LMW (%)
	40±2°C/75%±5 % RH (upright)	2 weeks	96.9	2.5	0.5	12.2	22.7	65.1	95.1	4.2
		4 weeks	96.1	3.6	0.3	14.1	31.3	54.6	94.4	5.7
	Freeze-thaw (-70°C to RT)	3 rounds	99.3	0.7	ND	8.9	12.9	78.2	97.3	2.6
		5 rounds	99.3	0.7	ND	8.9	14.3	76.8	97.3	2.7
	Shaking (200 rpm, protected from light, 25°C (upright))	3 days	99.2	0.8	ND	8.7	14.3	77.1	96.9	2.8
		5 days	99.2	0.8	ND	8.7	14.0	77.3	97.0	2.7

[0245] Insoluble particles: Results of insoluble particles showed that after storage for 4 weeks at 40±2°C/75%±5% RH (upright), the number of insoluble particles (< 25 μm) started to increase but remained at low levels. After storage for 3 months at 25±2°C/60%±5% RH (upright) and at 25±2°C/60%±5% RH (inverted), the number of insoluble particles (< 10 μm) started to increase but remained at low levels. After storage for at 2-8°C (upright) and 2-8°C (inverted) for 3 months, no significant changes in the number of insoluble particles were observed, which maintained at a low level. After 5 rounds of freezing and thawing (-70°C to RT) and 5 days of shaking (200 rpm, protected from light, 25°C, upright), the insoluble particles (< 5 μm) started to increase, but remained at a low level.

Table 18: Result of insoluble particles for formulation confirmation study

No.	Conditions	Sampling timepoint	MFI number/mL			
			≥2μm, <5μm	≥5μm, < 10μm	≥10μm	≥25μm
F1	N/A	T0	155	9	18	0
	2-8°C (upright)	3 months	280	10	10	0
	2-8°C (inverted)		221	33	0	0
		4 weeks	1042	45	30	0

No.	Conditions	Sampling timepoint	MFI number/mL			
			≥2μm, <5μm	≥5μm, < 10μm	≥10μm	≥25μm
	25±2°C/60%±5% RH (upright)	3 months	543	59	7	0
	25±2°C/60%±5% RH (inverted)	3 months	247	29	23	0
	40±2°C/75%±5% RH (upright)	4 weeks	1831	120	24	0
	Freeze-thaw (-70°C to RT)	5 rounds	438	0	15	0
	Shaking (200 rpm, protected from light, 25°C (upright))	5 days	616	0	11	0

[0246] Biological activity: No significant change in the biological activity was observed after storage for 4 weeks at 40±2°C/75%±5% RH (upright).

Table 19: Results of biological activity for formulation confirmation study

No.	Sampling timepoint	Biological activity
F1	40 ±2°C/75%±5%RH (upright) for 4 weeks	97%

[0247] Conclusions: The confirmation study was conducted for the following formulation: 150 mg/mL API, 20 mM glutamate/histidine buffer pH 5.3, 2.8% (w/v) proline and 0.02% (w/v) polysorbate 80. Data showed that the API had good physicochemical stability as well as conformational stability in this formulation at different tested conditions (2-8 °C (upright and inverted), accelerated (25±2°C/60%±5%RH upright and inverted), heated (40±2°C/75%±5%RH, upright), freeze-thaw (-70°C to RT), and shaking (200 rpm, protected from light, 25°C, upright). As such, this formulation was confirmed to support storage, transport, and clinical use of the API at high concentration.

5.8.4 Example 4: Formulation Analysis

[0248] A random batch of pharmaceutical composition as prepared by methods disclosed herein were subject to a series of assays for characterization and the results were provided below:

Table 20: Formulation characterization

Items	Exemplary Composition
pH	5.4

Osmolarity	346 mOsmol/Kg
Protein Concentration	151.4 mg/mL
Charge Variants (CEX)	
Main Peak	73.7%
Acidic Peaks	15.9%
Basic Peaks	10.4%
Purity (SEC)	
Main Peak%	98.9%
Total LMWS%	1.0%
Purity (CE-SDS; Reduced)	
Main Peak%	97.7%
Total LMWS%	1.3%
Purity (CE-SDS; Non-Reduced)	
Main Peak%	97.6%
HMWS%	2.0%
Polysorbate80 (w/v)	0.017%

5.8.5 Example 5: Toxicology and toxicokinetic profiling study

[0249] The toxicology and toxicokinetic (TK) profiles of the API were characterized in cynomolgus monkeys in a 28-day repeat SC dose study. The 150 mg/mL API solution for SC injection was formulated in 20 mM glutamic/histidine, 2.8% (w/v) proline, 0.02% (w/v) polysorbate 80, at pH 5.3, and supplied in 2R Type I glass vials with a 13 mm rubber stopper integrated into a plastic cap for clinical trial distribution. The formulation was a sterile, colorless to slightly yellow, slightly opalescent solution, essentially free of particles. The labelled volume was 1 mL per vial and there was an overfill of 0.22 mL per vial.

[0250] A GLP-compliant 28-day repeat dose toxicology and TK study was performed in cynomolgus monkeys to determine the toxicity and TK profile of the API by SC injection. Three doses (0, 38, and 240 mg/kg) were tested over a period of 28 days (a total of two doses on Days 1 and 22) in cynomolgus monkeys (3/sex/group). The local tolerance was also determined.

[0251] Following the first dose on Day 1, blood samples were collected at 0 (pre-dose), 1, 4, 24, 48, 96, 168, 216, 336, and 504 hours (before the second dose) post-dose from all animals.

[0252] Toxicology: No mortality occurred throughout the study. No test article related changes in clinical signs (including injection site), body weights, food consumption, body temperature, ophthalmic examinations, electrocardiograms, serum chemistry, coagulation, urinalysis, or macroscopic observations were noted in all tested groups.

[0253] TK: The median T_{max} was determined to be between 48.0 and 96.0 hours post-dose, and the median $T_{1/2}$ was between 279.2 to 379.5 hours. The systemic exposure (C_{max} and AUC) increased dose-proportionally without apparent sex differences. TK was evaluated following the first dose. The systemic exposure increased dose-proportionally without apparent sex differences.TK parameters were summarized in Table 21 below.

[0254] In summary, the NOAEL was determined to be 240 mg/kg in this study; the C_{max} and $AUC_{0\text{-}504h}$ following the first dose were 3010 μg/mL and 989000 h*μg/mL, respectively.

Table 21: TK parameters of the API after a single SC dose in cynomolgus monkeys

Dose (mg/kg)	Study Day	Sex	C_{max} (μg/mL)	T_{max} (h)	$AUC_{0\text{-}504h}$ (h*μg/mL)	$T_{1/2}$ (h)	Cl (mL/h/kg)	Vz (mL/kg)	$AUC_{0\text{-last}}$ (h*μg/mL)	$AUC_{0\text{-inf}}$ (h*μg/mL)
38	1	Male	494 ± 21	48.0 (24.0 - 96.0)	153000 ± 21000	315.4 (68.9 - 336.0)	0.196 ± 0.0771	56.9 ± 25.1	153000 ± 21000	212000 ± 70700
		Female	493± 69.6	48.0 (48.0 - 48.0)	164000 ± 28900	292.7 (278.3 - 411.4)	0.154 ± 0.0423	70.4 ± 9.35	164000 ± 28900	259000 ± 67900
		Mean	494 ± 46	48.0 (24.0 - 96.0)	159000 ± 23400	304.1 (68.9 - 411.4)	0.175 ± 0.0601	63.7 ± 18.5	159000 ± 23400	235000 ± 67000
240	1	Male	2960 ± 26.5	96.0 (48.0 - 96.0)	991000 ± 87100	279.2 (267.7 - 283.6)	0.170 ± 0.0211	67.7 ± 6.39	991000 ± 87100	1430000 ± 174000
		Female	3070 ± 452	48.0 (48.0 - 96.0)	988000 ± 94800	379.5 (260.8 - 472.6)	0.151 ± 0.0322	77.8 ± 10.1	988000 ± 94800	1640000 ± 382000
		Mean	3010 ± 292	72.0 (48.0 - 96.0)	989000 ± 81500	281.4 (260.8 - 472.6)	0.161 ± 0.0264	72.8 ± 9.34	989000 ± 81500	1530000 ± 290000

[0255] $AUC_{0\text{-last}}$= area under the serum concentration time curve from time 0 to the time of the last measured concentration; $AUC_{0\text{-inf}}$= area under the serum concentration time curve from time 0 to infinity; $AUC_{0\text{-504h}}$= area under the serum concentration time curve from time 0 to 504 hours; Cl =

clearance; C_{max}= maximum observed serum concentration; TK = toxicokinetic; $T_{1/2}$= half-life; T_{max}= time to maximum serum concentration; Vd= volume of distribution.

[0256] Data is presented as mean ± SD for C_{max}, AUC, Cl, Vd, and MRT values, and median (range) for T_{max} and $T_{1/2}$ values.

[0257] Local tolerance: The local tolerance of the API at the injection sites was evaluated macroscopically and microscopically in the 28-day SC repeat-dose toxicity study in cynomolgus monkeys. No compound-related signs of irritation at the infusion/injection site were noted up to the highest concentration tested (240 mg/kg/dose in SC study).

[0258] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims. Unless the context indicates otherwise, it is specifically intended that the various features described herein can be used in any combination. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Accordingly, the preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, *i.e.*, any elements developed that perform the same function, regardless of structure. Moreover, nothing disclosed herein is intended to be dedicated to the public regardless of whether such disclosure is explicitly recited in the claims.

[0259] All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission

that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

What is claimed is:

1. A pharmaceutical composition comprising

(A) an anti-OX40 antibody or antigen-binding fragment thereof comprising

(1) a light chain variable region (VL) comprising VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of SEQ ID NOs:1, 2, and 3, respectively; and

(2) a heavy chain variable region (VH) comprising VH CDR1, VL CDR2, and VH CDR3 having the amino acid sequences of SEQ ID NOs:4, 5 and 6, respectively; and

(B) a pharmaceutically acceptable carrier comprising a glutamic/histidine buffer, proline, and polysorbate 80.

2. The pharmaceutical composition of claim 1, wherein the anti-OX40 antibody or antigen-binding fragment is a chimeric antibody or antigen-binding fragment, a humanized antibody or antigen-binding fragment, or a human antibody or antigen-binding fragment.

3. The pharmaceutical composition of claim 1, wherein the anti-OX40 antibody or antigen-binding fragment is a humanized antibody or antigen-binding fragment.

4. The pharmaceutical composition of any one of claims 1 to 3, wherein
(1) the VL has at least 85%, at least 90%, at least 95%, at least 98%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:7; and/or
(2) the VH has at least 85%, at least 90%, at least 95%, at least 98%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:8.

5. The pharmaceutical composition of claim 4, wherein the VL has the amino acid sequence of SEQ ID NO:7, 9, or 10, and the VH has the amino acid sequence of SEQ ID NO:8, 11, or 12.

6. The pharmaceutical composition of claim 4, wherein the VL and VH have the amino acid sequences of SEQ ID NOs:7 and 8, respectively.

7. The pharmaceutical composition of any one of claims 1 to 6, wherein the anti-OX40 antibody or antigen-binding fragment is selected from the group consisting of a Fab, a Fab', a $F(ab')_2$, a Fv, a scFv, and a $(scFv)_2$.

8. The pharmaceutical composition of any one of claims 1 to 6, wherein the anti-OX40 antibody or antigen-binding fragment is an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody.

9. The pharmaceutical composition of claim 8, wherein the anti-OX40 antibody is a humanized

IgG1 antibody.

10. The pharmaceutical composition of claim 9, wherein the anti-OX40 antibody comprises a light chain constant region (CL) having at least 85% sequence identity to kappa CL (Cκ; SEQ ID NO:13) or lambda CL (Cλ; SEQ ID NO:14).

11. The pharmaceutical composition of claim 10, wherein the CL is Cκ (SEQ ID NO:13).

12. The pharmaceutical composition of any one of claims 9 to 11, wherein the anti-OX40 antibody comprises the wildtype heavy chain constant region (CH) of human IgG1 (SEQ ID NO:16), or a variant thereof having reduced binding to FcγR.

13. The pharmaceutical composition of claim 12, wherein the anti-OX40 antibody comprises the CH region of human IgG1 with N297A substitution.

14. The pharmaceutical composition of claim 9, wherein the anti-OX40 antibody comprises a light chain having the amino acid sequence of SEQ ID NO:19 and a heavy chain having the amino acid sequence of SEQ ID NO:20.

15. The pharmaceutical composition of any one of claims 1 to 14, comprising the anti-OX40 antibody or antigen-binding fragment at a concentration ranging from 80 to 180 mg/mL, from 100 to 180 mg/mL, from 120 to 180 mg/mL, from 140 to 180 mg/mL, from 100 to 160 mg/mL, or from 120 to 160 mg/mL.

16. The pharmaceutical composition of claim 15, comprising the antibody or antigen-binding fragment at a concentration ranging from 140 to 160 mg/mL.

17. The pharmaceutical composition of claim 15, comprising the antibody or antigen-binding fragment at a concentration of about 150 mg/mL.

18. The pharmaceutical composition of any one of claims 1 to 17, comprising the glutamic/histidine buffer at a concentration ranging from 10 to 50 mM, from 10 to 40 mM, from 10 to 30 mM, or from 15 to 25 mM.

19. The pharmaceutical composition of claim 18, comprising the glutamic/histidine buffer at 20 mM.

20. The pharmaceutical composition of any one of claims 1 to 19, comprising the proline at a concentration ranging from 1.0 to 5.0% (w/v), from 1.0 to 4.0% (w/v), from 1.0 to 3.0% (w/v), from 2.0 to 5.0% (w/v), from 2.0 to 4.0% (w/v), or from 2.0 to 3.0% (w/v).

21. The pharmaceutical composition of claim 20, comprising the proline at a concentration ranging from 2.5 to 3.0% (w/v).

22. The pharmaceutical composition of claim 20, comprising the proline at 2.8% (w/v).

23. The pharmaceutical composition of any one of claims 1 to 22, comprising the polysorbate 80 at a concentration ranging from 0.001 to 0.1% (w/v), from 0.005 to 0.05% (/v), or from 0.01 to 0.05% (v/v).

24. The pharmaceutical composition of claim 23, comprising the polysorbate 80 at a concentration ranging from 0.01 to 0.03% (w/v).

25. The pharmaceutical composition of claim 23, comprising the polysorbate 80 at 0.02% (w/v).

26. The pharmaceutical composition of any one of claims 1 to 25, having a pH ranging from 4.8 to 6.8, from 5.0 to 6.5, from 5.0 to 6.0, or from 5.0 to 5.8.

27. The pharmaceutical composition of claim 26, having a pH ranging from 5.1 to 5.6.

28. The pharmaceutical composition of claim 26, having a pH of about 5.3 or about 5.4.

29. The pharmaceutical composition of any one of claims 1 to 17, comprising 20 mM glutamic/histidine buffer, 2.8% (w/v) proline, and 0.01-0.03% (w/v) polysorbate 80, wherein the pH ranges from 5.0 to 5.6.

30. The pharmaceutical composition of any one of claims 1 to 17, comprising 20 mM glutamic/histidine buffer, 2.8% (w/v) proline, and 0.02% (w/v) polysorbate 80, wherein the pH is about 5.3 or about 5.4.

31. The pharmaceutical composition of any one of claims 1 to 30, wherein the pharmaceutical composition is in liquid form.

32. The pharmaceutical composition of any one of claims 1 to 30, wherein the pharmaceutical composition is lyophilized.

33. The pharmaceutical composition of any one of claims 1 to 32, wherein the viscosity of the pharmaceutical composition is no more than 20 cP, no more than 18 cP, no more than 16 cP, no more than 14 cP, no more than 12 cP, or no more than 10 cP.

34. The pharmaceutical composition of claim 33, wherein the viscosity is about 12 cP.

35. The pharmaceutical composition of any one of claims 1 to 34, wherein the osmotic pressure

of the pharmaceutical composition ranges from 200 to 400 mOsmol/kg, from 250 to 400 mOsmol/kg, or from 300 to 400 mOsmol/kg.

36. The pharmaceutical composition of claim 36, wherein the osmotic pressure is about 350 mOsmol/kg.

37. The pharmaceutical composition of any one of claims 1 to 36, wherein the purity of the antibody or antigen-binding fragment is at least 90%.

38. The pharmaceutical composition of claim 37, wherein the purity of the antibody or antigen-binding fragment is 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, or at least 99%.

39. The pharmaceutical composition of claim 37 or 38, wherein the purity of the antibody or antigen-binding fragment is measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC) or Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS).

40. The pharmaceutical composition of claim 39, wherein the purity of the antibody or antigen-binding fragment is at least 95% measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC).

41. The pharmaceutical composition of claim 39, wherein the purity of the antibody or antigen-binding fragment is at least 90% measured by Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS).

42. The pharmaceutical composition of any one of claims 1 to 41, comparing no more than 60% charge variants.

43. The pharmaceutical composition of claim 42, comprising no more than 55%, no more than 50%, no more than 45%, or no more than 40%, no more than 35%, or no more than 30% charge variants.

44. The pharmaceutical composition of claim 42 or 43, wherein the charge variants are measured by Cation Exchange-High Performance Liquid Chromatography (CEX-HPLC).

45. The pharmaceutical composition of any one of claims 1 to 44, wherein the purity of the antibody or antigen-binding fragment is reduced by no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no

more than 3%, or no more than 2% after storage for a period of time that is at least 2 weeks.

46. The pharmaceutical composition of claim 45, wherein the purity is measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC) or Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS).

47. The pharmaceutical composition of any one of claims 1 to 46, wherein the charge variants of the antibody or antigen-binding fragment are increased by no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5% after storage for a period of time that is at least 2 weeks.

48. The pharmaceutical composition of claim 47, wherein the charge variants are measured by Cation Exchange-High Performance Liquid Chromatography (CEX-HPLC).

49. The pharmaceutical composition of any one of claims 45 to 48, wherein the period of time is at least 2 weeks, at least 4 weeks, at least 2 months, at least 3 months, at least 6 months, at least 9 months, at least 12 months, at least 18 months, or at least 24 months.

50. The pharmaceutical composition of any one of claims 45 to 49, that is stored at about -80°C, about -20°C, about 4°C, about 25°C or about 40°C.

51. The pharmaceutical composition of any one of claims 45 to 50, that is stored upright or inverted.

52. The pharmaceutical composition of any one of claims 45 to 51, that is stored with agitation.

53. The pharmaceutical composition of any one of claims 1 to 52, wherein the purity of the antibody or antigen-binding fragment is reduced by no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3%, or no more than 2% after at least 1, at least 2, at least 3, at least 4, or at least 5 freeze-thaw cycles.

54. The pharmaceutical composition of claim 53, wherein the purity is measured by Size Exclusion-Ultra High Performance Liquid Chromatography (SEC-UPLC) or Sodium Dodecyl Sulfate-Polyacrylamide Capillary Electrophoresis (CE-SDS).

55. The pharmaceutical composition of any one of claims 1 to 54, wherein the charge variants of the antibody or antigen-binding fragment are increased to no more than 60%, to no more than 55%, no more than 50%, no more than 45%, or no more than 40%, or no more than 35% after

at least 1, at least 2, at least 3, at least 4, or at least 5 freeze-thaw cycles.

56. The pharmaceutical composition of claim 55, wherein the charge variants are measured by Cation Exchange-High Performance Liquid Chromatography (CEX-HPLC).

57. The pharmaceutical composition of any one of claims 1 to 56 that is suitable for subcutaneous injection.

58. A method of treating an OX40-associated disease comprising administering a therapeutically effective amount of the pharmaceutical composition of any one of claims 1 to 57.

59. The method of claim 58 comprising subcutaneous injection.

60. Use of the pharmaceutical composition of any one of claims 1 to 57 in treating an OX40-associated disease.

61. Use of the pharmaceutical composition of any one of claims 1 to 57 in preparation of a medication for treating an OX40-associated disease.

62. The method or use of any one of claims 58 to 61, wherein the OX40-associated disease is alopecia areata (AA) or atopic dermatitis (AD).

63. A kit comprising the pharmaceutical composition of any one of claims 1 to 57.