ANTI-SIGLEC-8 ANTIBODY FORMULATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 63/104,436, filed October 22, 2020, the disclosure of which is incorporated herein by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 701712001340SEQLIST.TXT, date recorded: October 13, 2021, size: 93,125 bytes).

FIELD OF THE INVENTION

[0003] The present disclosure relates to pharmaceutical compositions (*e.g.*, liquid formulations) comprising a monoclonal antibody that binds to a human Siglec-8 for subcutaneous administration, as well as articles of manufacture related thereto.

BACKGROUND

[0004] Siglec-8 is a sialic acid-binding immunoglobulin-like lectin expressed specifically by eosinophils, mast cells, and basophils. Along with mast cells, eosinophils can promote an inflammatory response that plays a beneficial functional role such as controlling an infection at a specific tissue site. Several diseases have been shown to be linked to eosinophil activation such as Churg Strauss syndrome, rheumatoid arthritis, and allergic asthma (Wechsler et al., *J Allergy Clin Immunol*., 2012, 130(3):563-71). There is currently a need for therapies that can control the activity of immune cells involved in inflammation, such as the activity of eosinophils and mast cells. Antibodies recognizing human Siglec-8 are described in U.S. Pat. No. 8,207,305, U.S. Pat. No. 8,197,811, U.S. Pat. No. 7,871,612, and U.S. Pat. No. 7,557,191. Humanized anti-Siglec-8 antibodies are described in U.S. Pat. No. 9,546,215.

[0005] Formulating an antibody for commercial use requires identification of a combination of buffer, pH, and optional excipients that allows for product stability and solubility. Subcutaneous administration further requires that the formulation keep the product from aggregating at high concentration, which can lead to formation of large accumulations of product that clog needles and/or require larger gauge needles, which can increase the pain or discomfort associated with administration. Current commercial products at high concentrations (*e.g.*, for subcutaneous administration) are typically formulated in histidine buffers with pH conditions of 5.5-6.3 and

sugar concentrations of 5-9%, with further optional excipients. A formulation that is successful for commercial use should be able to maintain a high concentration of antibody in solution for a given period of time with little to no increase in product turbidity or aggregate formation.

[0006] Without wishing to be bound to theory, it is thought that subcutaneous administration of anti-Siglec-8 antibodies may be advantageous, *e.g.*, in reducing rate and/or severity of administration-related reactions. Therefore, the need exists for liquid formulations suitable for subcutaneous administration of anti-Siglec-8 antibodies that allow for long-term stability and solubility of the antibody.

[0007] All references cited herein, including patent applications, patent publications, and scientific literature, are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

BRIEF SUMMARY

[0008] To meet this and other needs, the present disclosure relates, *inter alia*, to pharmaceutical compositions (*e.g.*, liquid formulations) and kits comprising a monoclonal antibody that binds to a human Siglec-8 for subcutaneous administration. These are based at least in part on the demonstration herein that the solubility of certain anti-Siglec-8 antibodies is dependent upon pH and specific buffer composition. Surprisingly, a salting out effect was observed in the presence of arginine at high concentration, and also observed in the presence of sodium chloride. However, other combinations of buffer, pH, and optional sugar excipient provided for high antibody concentrations necessary for subcutaneous formulations with acceptable solubility and long-term stability.

[0009] Accordingly, certain aspects of the present disclosure relate to pharmaceutical compositions (*e.g.*, liquid formulations) comprising a monoclonal antibody that binds to a human Siglec-8 and histidine or sodium acetate in a concentration of about 10mM to about 25mM. In some embodiments, the pH of the liquid formulation is between 5.0 and 6.3. In some embodiments, the antibody comprises: (1) a heavy chain variable region comprising: an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62; an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and (1) a light chain variable region comprising: an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64; an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66. In some embodiments, the antibody is in a concentration of about 70mg/mL to about 210mg/mL. In

some embodiments, the antibody is in a concentration of about 100mg/mL to about 200mg/mL. In some embodiments, the antibody is in a concentration of about 125mg/mL to about 175mg/mL. In some embodiments, the antibody is in a concentration of about 135mg/mL to about 165mg/mL. In some embodiments, the antibody is in a concentration of about 150mg/mL.

[0010] In some embodiments, the formulation comprises L-histidine or L-histidine hydrochloride in a concentration of about 15mM. In some embodiments, the pH of the liquid formulation is 6.0.

[0011] In some embodiments, the formulation comprises sodium acetate in a concentration of about 15mM. In some embodiments, the pH of the liquid formulation is about 5.2 to about 5.8. In some embodiments, the pH of the liquid formulation is 5.5.

[0012] In some embodiments, the formulation comprises sucrose in a concentration of about 5% to about 9%. In some embodiments, the formulation comprises sucrose in a concentration of about 5% to about 7.5%. In some embodiments, the formulation comprises sucrose in a concentration of about 5%.

[0013] In some embodiments, the formulation comprises trehalose in a concentration of about 4% to about 10%. In some embodiments, the formulation comprises trehalose in a concentration of about 5% to about 7.5%. In some embodiments, the formulation comprises trehalose in a concentration of 6.6%. In some embodiments, the trehalose is trehalose dihydrate.

[0014] In some embodiments according to any of the embodiments described herein, the formulation comprises polysorbate 80 in a concentration of about 0.0225% to about 0.0275% (w/v). In some embodiments, the polysorbate 80 is in a concentration of about 0.025% (w/v).

[0015] In some embodiments, the formulation comprises the antibody that binds to a human Siglec-8 in a concentration of 150mg/mL; 15mM L-histidine or L-histidine hydrochloride; 175mM trehalose dihydrate; and 0.025% polysorbate 80 (w/v); wherein the pH of the liquid formulation is 6.0. In some embodiments, the formulation comprises the antibody that binds to a human Siglec-8 in a concentration of 150mg/mL; 15mM sodium acetate; 175mM trehalose dihydrate; and 0.025% polysorbate 80 (w/v); wherein the pH of the liquid formulation is 5.5. In some embodiments, the formulation comprises the antibody that binds to a human Siglec-8 in a concentration of 150mg/mL; 15mM L-histidine or L-histidine hydrochloride; 5% sucrose; and 0.025% polysorbate 80 (w/v); wherein the pH of the liquid formulation is 6.0.

[0016] In some embodiments according to any of the embodiments described herein, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ

ID NO:6; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:16 or 21. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:16. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:21. In some embodiments, the antibody comprises a heavy chain Fc region comprising a human IgG Fc region. In some embodiments, the human IgG Fc region comprises a human IgG1 Fc region. In some embodiments, the human IgG1 Fc region is non-fucosylated. In some embodiments, less than about 50% of the N-linked glycans attached to the Fc region of the antibodies in the formulation comprise fucose. In some embodiments, the human IgG Fc region comprises a human IgG4 Fc region. In some embodiments, the human IgG4 Fc region comprises the amino acid substitution S228P, wherein the amino acid residues are numbered according to the EU index as in Kabat. In some embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:75; and a light chain comprising the amino acid sequence of SEQ ID NO:76 or 77. In some embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:75 and a light chain comprising the amino acid sequence of SEQ ID NO:76. In some embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:75 and a light chain comprising the amino acid sequence of SEQ ID NO:77. In some embodiments, the antibody has been engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In some embodiments, at least one or two of the heavy chains of the antibody is non-fucosylated.

[0017] Other aspects of the present disclosure relate to articles of manufacture or kits comprising a container enclosing the formulation of any one of the embodiments described herein. In some embodiments, the container is a glass vial. In some embodiments, the articles of manufacture or kits further comprise instructions for administering the formulation subcutaneously.

[0018] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present disclosure. These and other aspects of the present disclosure will become apparent to one of skill in the art. These and other embodiments of the present disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **FIG. 1** shows turbidity transition during antibody concentration in 15mM potassium phosphate buffer at pH 7.2. Shown is the turbidity of the anti-Siglec-8 antibody formulation at 17.0mg/mL (left) or 110.0mg/mL (right) antibody.

[0020] **FIG. 2** shows turbidity transition during antibody concentration in 15mM L-histidine buffer at pH 6.4. Shown is the turbidity of the anti-Siglec-8 antibody formulation at 11.0mg/mL (left) or 185.0mg/mL (right) antibody.

[0021] **FIG. 3** shows turbidity transition during antibody concentration in 15mM sodium succinate buffer at pH 6.0. Shown is the turbidity of the anti-Siglec-8 antibody formulation at 18.0mg/mL (left) or 170.0mg/mL (right) antibody.

[0022] **FIG. 4** shows turbidity transition during antibody concentration in 15mM sodium succinate buffer at pH 5.6. Shown is the turbidity of the anti-Siglec-8 antibody formulation at 10.0mg/mL (left) or 165.0mg/mL (right) antibody.

[0023] **FIG. 5** shows turbidity transition during antibody concentration in 15mM sodium acetate buffer at pH 5.0. Shown is the turbidity of the anti-Siglec-8 antibody formulation at 17.0mg/mL (left) or 190.0mg/mL (right) antibody.

[0024] **FIG. 6** shows comparison of turbidity of anti-Siglec-8 antibody formulations with various excipients. All samples used 15mM potassium phosphate buffer at pH 7.2. Shown are (left to right): 110mg/mL antibody in control formulation, 85mg/mL antibody in formulation with 320mM arginine, 70mg/mL antibody in formulation with 540mM sucrose, and 80mg/mL antibody in formulation with 500mM sodium chloride.

[0025] **FIG. 7** shows comparison of turbidity of anti-Siglec-8 antibody formulations with various excipients. All samples used 15mM histidine buffer at pH 6.4. Shown are (left to right): 185mg/mL antibody in control formulation, 165mg/mL antibody in formulation with 100mM arginine, 150mg/mL antibody in formulation with 260mM sucrose, and 165mg/mL antibody in formulation with 140mM sodium chloride.

[0026] **FIG. 8** shows comparison of turbidity of anti-Siglec-8 antibody formulations with various excipients. All samples used 15mM sodium succinate buffer at pH 5.6. Shown are (left to right): 165mg/mL antibody in control formulation, 150mg/mL antibody in formulation with 100mM arginine, 130mg/mL antibody in formulation with 260mM sucrose, and 150mg/mL antibody in formulation with 140mM sodium chloride.

[0027] **FIG. 9** shows results from a phase 1 study of subcutaneous administration of anti-Siglec-8 antibody. Healthy volunteers were dosed according to the following cohorts: placebo administered SC, 0.3 mg/kg anti-Siglec-8 administered SC, 1.0 mg/kg anti-Siglec-8 administered SC, 3.0 mg/kg anti-Siglec-8 administered SC, 5.0 mg/kg anti-Siglec-8 administered SC, 300 mg anti-Siglec-8 administered SC, 1.0 mg/kg anti-Siglec-8 administered IV, and 3.0 mg/kg anti-Siglec-8 administered IV. Number of volunteers in each cohort is indicated by n. For all cohorts, median amount of blood eosinophils ($x10^3$/mL) was measured at baseline, 1 hr post administration, 3 hr post administration, Day 15 post administration, Day 35 post administration, Day 56 post administration, and Day 85 post administration. SC: subcutaneous injection; IV: intravenous infusion; PBO: placebo.

DETAILED DESCRIPTION

I. Definitions

[0028] It is to be understood that the present disclosure is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a molecule" optionally includes a combination of two or more such molecules, and the like.

[0029] The term “about” as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0030] It is understood that aspects and embodiments of the present disclosure include “comprising,” “consisting,” and “consisting essentially of” aspects and embodiments.

[0031] The term “antibody” includes polyclonal antibodies, monoclonal antibodies (including full length antibodies which have an immunoglobulin Fc region), antibody compositions with polyepitopic specificity, multispecific antibodies (*e.g.*, bispecific antibodies, diabodies, and single-chain molecules), as well as antibody fragments (*e.g.*, Fab, $F(ab')_2$, and Fv). The term “immunoglobulin” (Ig) is used interchangeably with “antibody” herein.

[0032] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called a J chain, and contains 10 antigen binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ε isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_H1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see *e.g.*, *Basic and Clinical Immunology*, 8th Edition, Daniel P. Sties, Abba I. Terr and Tristram G. Parsolw (eds), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

[0033] The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α, δ, ε, γ and μ, respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in the CH sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. IgG1 antibodies can exist in multiple polymorphic variants termed allotypes (reviewed in Jefferis and Lefranc 2009. mAbs Vol 1 Issue 4 1-7) any of which are suitable for use in the present disclosure. Common allotypic variants in human populations are those designated by the letters a, f, n, z.

[0034] An "isolated" antibody is one that has been identified, separated and/or recovered from a component of its production environment (*e.g.*, naturally or recombinantly). In some embodiments, the isolated polypeptide is free of association with all other components from its

production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In some embodiments, the polypeptide is purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated polypeptide or antibody is prepared by at least one purification step.

[0035] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. In some embodiments, monoclonal antibodies have a C-terminal cleavage at the heavy chain and/or light chain. For example, 1, 2, 3, 4, or 5 amino acid residues are cleaved at the C-terminus of heavy chain and/or light chain. In some embodiments, the C-terminal cleavage removes a C-terminal lysine from the heavy chain. In some embodiments, monoclonal antibodies have an N-terminal cleavage at the heavy chain and/or light chain. For example, 1, 2, 3, 4, or 5 amino acid residues are cleaved at the N-terminus of heavy chain and/or light chain. In some embodiments, monoclonal antibodies are highly specific, being directed against a single antigenic site. In some embodiments, monoclonal antibodies are highly specific, being directed against multiple antigenic sites (such as a bispecific antibody or a multispecific antibody). The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by a variety of techniques, including, for example, the hybridoma method, recombinant DNA methods, phage-display technologies, and technologies for producing human or human-like antibodies in animals that

have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences.

[0036] The term "naked antibody" refers to an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

[0037] The terms "full-length antibody," "intact antibody" or "whole antibody" are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. Specifically whole antibodies include those with heavy and light chains including an Fc region. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0038] An "antibody fragment" comprises a portion of an intact antibody, the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870, Example 2; Zapata *et al., Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0039] Papain digestion of antibodies produced two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V_H), and the first constant domain of one heavy chain (C_H1). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large $F(ab')_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the C_H1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0040] The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

[0041] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0042] "Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. In some embodiments, the sFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0043] "Functional fragments" of the antibodies of the present disclosure comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the Fv region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0044] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is (are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; Morrison *et al., Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Chimeric antibodies of interest herein include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with an antigen of interest. As used herein, "humanized antibody" is used as a subset of "chimeric antibodies."

[0045] "Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, *etc*. In some embodiments, the number of these amino acid substitutions in the FR are no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al., Nature* 321:522-525 (1986); Riechmann *et al., Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409. In some embodiments, humanized antibodies are directed against a single antigenic site. In some embodiments, humanized antibodies are directed against multiple antigenic sites. An alternative humanization method is described in U.S. Pat. No. 7,981,843 and U.S. Patent Application Publication No. 2006/0134098.

[0046] The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as "VH" and "VL", respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.

[0047] The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody-variable domain that are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, *e.g.*, Xu *et al. Immunity* 13:37-45 (2000); Johnson and Wu in *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003)). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, *e.g.*, Hamers-Casterman *et al., Nature* 363:446-448 (1993) and Sheriff *et al.*, *Nature Struct. Biol.* 3:733-736 (1996).

[0048] A number of HVR delineations are in use and are encompassed herein. The HVRs that are Kabat complementarity-determining regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al., Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institute of Health, Bethesda, MD (1991)). Chothia HVRs refer instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

Loop	Kabat	Chothia	Contact
L1	L24-L34	L26-L34	L30-L36
L2	L50-L56	L50-L56	L46-L55
L3	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H32	H30-H35B (Kabat Numbering)
H1	H31-H35	H26-H32	H30-H35 (Chothia Numbering)
H2	H50-H65	H53-H56	H47-H58
H3	H95-H102	H95-H102	H93-H101

[0049] Unless otherwise indicated, the variable-domain residues (HVR residues and framework region residues) are numbered according to Kabat *et al., supra.*

[0050] "Framework" or "FR" residues are those variable-domain residues other than the HVR residues as herein defined.

[0051] The expression "variable-domain residue-numbering as in Kabat" or "amino-acid-position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat *et al.*, *supra.* Using this numbering system, the actual linear amino acid sequence may

contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, *etc*. according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0052] An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework or a human consensus framework. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less.

[0053] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the

length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

[0054] An antibody that "binds to", "specifically binds to" or is "specific for" a particular a polypeptide or an epitope on a particular polypeptide is one that binds to that particular polypeptide or epitope on a particular polypeptide without substantially binding to any other polypeptide or polypeptide epitope. In some embodiments, binding of an anti-Siglec-8 antibody described herein (*e.g.*, an antibody that binds to human Siglec-8) to an unrelated non-Siglec-8 polypeptide is less than about 10% of the antibody binding to Siglec-8 as measured by methods known in the art (*e.g.*, enzyme-linked immunosorbent assay (ELISA)). In some embodiments, an antibody that binds to a Siglec-8 (*e.g.*, an antibody that binds to human Siglec-8) has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 2 nM, $\leq$ 1 nM, $\leq$ 0.7 nM, $\leq$0 .6 nM, $\leq$ 0.5 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

[0055] The term "anti-Siglec-8 antibody" or "an antibody that binds to human Siglec-8" refers to an antibody that binds to a polypeptide or an epitope of human Siglec-8 without substantially binding to any other polypeptide or epitope of an unrelated non-Siglec-8 polypeptide.

[0056] The term "Siglec-8" as used herein refers to a human Siglec-8 protein. The term also includes naturally occurring variants of Siglec-8, including splice variants or allelic variants. The amino acid sequence of an exemplary human Siglec-8 is shown in SEQ ID NO:72. The amino acid sequence of another exemplary human Siglec-8 is shown in SEQ ID NO:73. In some embodiments, a human Siglec-8 protein comprises the human Siglec-8 extracellular domain fused to an immunoglobulin Fc region. The amino acid sequence of an exemplary human Siglec-8 extracellular domain fused to an immunoglobulin Fc region is shown in SEQ ID NO:74. The amino acid sequence underlined in SEQ ID NO:74 indicates the Fc region of the Siglec-8 Fc fusion protein amino acid sequence.

Human Siglec-8 Amino Acid Sequence

GYLLQVQELVTVQEGLCVHVPCSFSYPQDGWTDSDPVHGYWFRAGDRPYQDAPVATN
NPDREVQAETQGRFQLLGDIWSNDCSLSIRDARKRDKGSYFFRLERGSMKWSYKSQLN
YKTKQLSVFVTALTHRPDILILGTLESGHSRNLTCSVPWACKQGTPPMISWIGASVSSPG
PTTARSSVLTLTPKPQDHGTSLTCQVTLPGTGVTTTSTVRLDVSYPPWNLTMTVFQGDA
TASTALGNGSSLSVLEGQSLRLVCAVNSNPPARLSWTRGSLTLCPSRSSNPGLLELPRVH

VRDEGEFTCRAQNAQGSQHISLSLSLQNEGTGTSRPVSQVTLAAVGGAGATALAFLSFC
IIFIIVRSCRKKSARPAAGVGDTGMEDAKAIRGSASQGPLTESWKDGNPLKKPPPAVAPS
SGEEGELHYATLSFHKVKPQDPQGQEATDSEYSEIKIHKRETAETQACLRNHNPSSKEV
RG (SEQ ID NO:72)

Human Siglec-8 Amino Acid Sequence

GYLLQVQELVTVQEGLCVHVPCSFSYPQDGWTDSDPVHGYWFRAGDRPYQDAPVATN
NPDREVQAETQGRFQLLGDIWSNDCSLSIRDARKRDKGSYFFRLERGSMKWSYKSQLN
YKTKQLSVFVTALTHRPDILILGTLESGHPRNLTCSVPWACKQGTPPMISWIGASVSSPG
PTTARSSVLTLTPKPQDHGTSLTCQVTLPGTGVTTTSTVRLDVSYPPWNLTMTVFQGDA
TASTALGNGSSLSVLEGQSLRLVCAVNSNPPARLSWTRGSLTLCPSRSSNPGLLELPRVH
VRDEGEFTCRAQNAQGSQHISLSLSLQNEGTGTSRPVSQVTLAAVGGAGATALAFLSFC
IIFIIVRSCRKKSARPAAGVGDTGMEDAKAIRGSASQGPLTESWKDGNPLKKPPPAVAPS
SGEEGELHYATLSFHKVKPQDPQGQEATDSEYSEIKIHKRETAETQACLRNHNPSSKEV
RG (SEQ ID NO:73)

Siglec-8 Fc Fusion Protein Amino Acid Sequence

GYLLQVQELVTVQEGLCVHVPCSFSYPQDGWTDSDPVHGYWFRAGDRPYQDAPVATN
NPDREVQAETQGRFQLLGDIWSNDCSLSIRDARKRDKGSYFFRLERGSMKWSYKSQLN
YKTKQLSVFVTALTHRPDILILGTLESGHSRNLTCSVPWACKQGTPPMISWIGASVSSPG
PTTARSSVLTLTPKPQDHGTSLTCQVTLPGTGVTTTSTVRLDVSYPPWNLTMTVFQGDA
TASTALGNGSSLSVLEGQSLRLVCAVNSNPPARLSWTRGSLTLCPSRSSNPGLLELPRVH
VRDEGEFTCRAQNAQGSQHISLSLSLQNEGTGTSRPVSQVTLAAVGGIEGRSDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:74)

[0057] Antibodies that "induce apoptosis" or are "apoptotic" are those that induce programmed cell death as determined by standard apoptosis assays, such as binding of annexin

V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). For example, the apoptotic activity of the anti-Siglec-8 antibodies (*e.g.*, an antibody that binds to human Siglec-8) of the present disclosure can be shown by staining cells with annexin V.

[0058] Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptors); and B cell activation.

[0059] "Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.*, natural killer (NK) cells, neutrophils and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are required for killing of the target cell by this mechanism. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Fc expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991). In some embodiments, an anti-Siglec-8 antibody (*e.g.*, an antibody that binds to human Siglec-8) described herein enhances ADCC. To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al.*, *PNAS USA* 95:652-656 (1998). Other Fc variants that alter ADCC activity and other antibody properties include those disclosed by Ghetie et al., Nat Biotech. 15:637-40, 1997; Duncan et al, Nature 332:563-564, 1988; Lund et al., J. Immunol 147:2657-2662, 1991; Lund et al, Mol Immunol 29:53-59, 1992; Alegre et al, Transplantation 57:1537-1543, 1994; Hutchins et al., Proc Natl. Acad Sci USA 92:11980-11984, 1995; Jefferis et al, Immunol Lett. 44:111-117, 1995; Lund et al., FASEB J9:115-119, 1995; Jefferis et al, Immunol Lett 54:101-104, 1996; Lund et al, J Immunol 157:4963-4969, 1996; Armour et al., Eur J Immunol 29:2613-2624, 1999; Idusogie et al, J Immunol 164:4178-4184, 200; Reddy et al, J Immunol 164:1925-1933, 2000;

Xu et al., Cell Immunol 200:16-26, 2000; Idusogie et al, J Immunol 166:2571-2575, 2001; Shields et al., J Biol Chem 276:6591-6604, 2001; Jefferis et al, Immunol Lett 82:57-65. 2002; Presta et al., Biochem Soc Trans 30:487-490, 2002; Lazar et al., Proc. Natl. Acad. Sci. USA 103:4005-4010, 2006; U.S. Pat. Nos. 5,624,821; 5,885,573; 5,677,425; 6,165,745; 6,277,375; 5,869,046; 6,121,022; 5,624,821; 5,648,260; 6,194,551; 6,737,056; 6,821,505; 6,277,375; 7,335,742; and 7,317,091.

[0060] The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. Suitable native-sequence Fc regions for use in the antibodies of the present disclosure include human IgG1, IgG2, IgG3 and IgG4. A single amino acid substitution (S228P according to Kabat numbering; designated IgG4Pro) may be introduced to abolish the heterogeneity observed in recombinant IgG4 antibody. *See* Angal, S. et al. (1993) Mol Immunol 30, 105–108.

[0061] “Non-fucosylated” or “fucose-deficient” antibody refers to a glycosylation antibody variant comprising an Fc region wherein a carbohydrate structure attached to the Fc region has reduced fucose or lacks fucose. In some embodiments, an antibody with reduced fucose or lacking fucose has improved ADCC function. Non-fucosylated or fucose-deficient antibodies have reduced fucose relative to the amount of fucose on the same antibody produced in a cell line. In some embodiments, a non-fucosylated or fucose-deficient antibody composition contemplated herein is a composition wherein less than about 50% of the N-linked glycans attached to the Fc region of the antibodies in the composition comprise fucose.

[0062] The terms "fucosylation" or “fucosylated” refers to the presence of fucose residues within the oligosaccharides attached to the peptide backbone of an antibody. Specifically, a fucosylated antibody comprises α (l,6)-linked fucose at the innermost N-acetylglucosamine (GlcNAc) residue in one or both of the N-linked oligosaccharides attached to the antibody Fc region, e.g. at position Asn 297 of the human IgG1 Fc domain (EU numbering of Fc region residues). Asn297 may also be located about + 3 amino acids upstream or downstream of position 297, i.e. between positions 294 and 300, due to minor sequence variations in immunoglobulins.

[0063] The "degree of fucosylation" is the percentage of fucosylated oligosaccharides relative to all oligosaccharides identified by methods known in the art *e.g.*, in an N-glycosidase F treated antibody composition assessed by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS). In a composition of a "fully fucosylated antibody" essentially all oligosaccharides comprise fucose residues, *i.e.* are fucosylated. In some embodiments, a composition of a fully fucosylated antibody has a degree of fucosylation of at least about 90%. Accordingly, an individual antibody in such a composition typically comprises fucose residues in each of the two N-linked oligosaccharides in the Fc region. Conversely, in a composition of a "fully non-fucosylated" antibody essentially none of the oligosaccharides are fucosylated, and an individual antibody in such a composition does not contain fucose residues in either of the two N-linked oligosaccharides in the Fc region. In some embodiments, a composition of a fully non-fucosylated antibody has a degree of fucosylation of less than about 10%. In a composition of a "partially fucosylated antibody" only part of the oligosaccharides comprise fucose. An individual antibody in such a composition can comprise fucose residues in none, one or both of the N-linked oligosaccharides in the Fc region, provided that the composition does not comprise essentially all individual antibodies that lack fucose residues in the N-linked oligosaccharides in the Fc region, nor essentially all individual antibodies that contain fucose residues in both of the N- linked oligosaccharides in the Fc region. In one embodiment, a composition of a partially fucosylated antibody has a degree of fucosylation of about 10% to about 80% (*e.g.*, about 50% to about 80%, about 60% to about 80%, or about 70% to about 80%).

[0064] “Binding affinity” as used herein refers to the strength of the non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). In some embodiments, the binding affinity of an antibody for a Siglec-8 (which may be a dimer, such as the Siglec-8-Fc fusion protein described herein) can generally be represented by a dissociation constant (*Kd*). Affinity can be measured by common methods known in the art, including those described herein.

[0065] “Binding avidity” as used herein refers to the binding strength of multiple binding sites of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen).

[0066] An “isolated” nucleic acid molecule encoding the antibodies herein is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. In some embodiments, the isolated nucleic acid is free of association with all components associated with

the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies herein existing naturally in cells.

[0067] The term "pharmaceutical formulation" refers to a preparation that is in such form as to permit the biological activity of the active ingredient to be effective, and that contains no additional components that are unacceptably toxic to an individual to which the formulation would be administered. Such formulations are sterile. In some embodiments, the pharmaceutical formulation is a liquid formulation.

[0068] As used herein, the term "treatment" or "treating" refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. An individual is successfully "treated", for example, if one or more symptoms associated with a disease are mitigated or eliminated. For example, an individual is successfully "treated" if treatment results in increasing the quality of life of those suffering from a disease, decreasing the dose of other medications required for treating the disease, reducing the frequency of recurrence of the disease, lessening severity of the disease, delaying the development or progression of the disease, and/or prolonging survival of individuals.

[0069] As used herein, "in conjunction with" or "in combination with" refers to administration of one treatment modality in addition to another treatment modality. As such, "in conjunction with" or "in combination with" refers to administration of one treatment modality before, during or after administration of the other treatment modality to the individual.

[0070] As used herein, the term "prevention" or "preventing" includes providing prophylaxis with respect to occurrence or recurrence of a disease in an individual. An individual may be predisposed to a disease, susceptible to a disease, or at risk of developing a disease, but has not yet been diagnosed with the disease. In some embodiments, anti-Siglec-8 antibodies (*e.g.*, an antibody that binds to human Siglec-8) described herein are used to delay development of a disease.

[0071] As used herein, an individual "at risk" of developing a disease may or may not have detectable disease or symptoms of disease, and may or may not have displayed detectable disease or symptoms of disease prior to the treatment methods described herein. "At risk"

denotes that an individual has one or more risk factors, which are measurable parameters that correlate with development of the disease, as known in the art. An individual having one or more of these risk factors has a higher probability of developing the disease than an individual without one or more of these risk factors.

[0072] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0073] As used herein, an "individual" or a "subject" is a mammal. A "mammal" for purposes of treatment includes humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, *etc.* In some embodiments, the individual or subject is a human.

II. Compositions

[0074] In some aspects, also provided herein are compositions (e.g., pharmaceutical compositions, such as liquid formulations) comprising any of the anti-Siglec-8 antibodies described herein (e.g., an antibody that binds to Siglec-8). In some embodiments, the composition is for subcutaneous administration. In some embodiments, provided herein is a liquid formulation comprising: (a) a monoclonal antibody that binds to a human Siglec-8, wherein the antibody is in a concentration of about 70mg/mL to about 210mg/mL; and (b) histidine or sodium acetate in a concentration of about 15mM. In some embodiments, the pH of the liquid formulation is between 5.0 and 6.3.

[0075] In some embodiments, the antibody comprises: (1) a heavy chain variable region comprising: an HVR-H1 comprising the amino acid sequence of SEQ ID NO:1; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:2; an HVR-H3 comprising the amino acid sequence of SEQ ID NO:3; and (1) a light chain variable region comprising: an HVR-L1 comprising the amino acid sequence of SEQ ID NO:4; an HVR-L2 comprising the amino acid sequence of SEQ ID NO:5; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:16 or 21. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and a light

chain variable region comprising the amino acid sequence of SEQ ID NO:16. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:21. In some embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:75; and/or a light chain comprising the amino acid sequence of SEQ ID NO:76 or 77. In some embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:75; and a light chain comprising the amino acid sequence of SEQ ID NO:76. In some embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:75; and a light chain comprising the amino acid sequence of SEQ ID NO:77.

[0076] In some embodiments, the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein less than about 50% of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some embodiments, at least one or two of the heavy chains of the antibody is non-fucosylated. In some embodiments, substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some embodiments, the Fc region is a human IgG Fc region (*e.g.*, human IgG1 or IgG4 as described herein).

[0077] Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington: The Science and Practice of Pharmacy, 20th Ed., Lippincott Williams & Wiklins, Pub., Gennaro Ed., Philadelphia, Pa. 2000). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers, antioxidants including ascorbic acid, methionine, Vitamin E, sodium metabisulfite; preservatives, isotonicifiers, stabilizers, metal complexes (e.g., Zn-protein complexes); chelating agents such as EDTA and/or non-ionic surfactants. Exemplary formulations are described herein.

[0078] The instant application demonstrates formulations suitable for an anti-Siglec-8 antibody at high concentrations sufficient for subcutaneous administration without turbidity or gel formation. In some embodiments, the antibody is in a concentration of about 70mg/mL to about 210mg/mL, about 70mg/mL to about 175mg/mL, about 90mg/mL to about 210mg/mL, about 100mg/mL to about 210mg/mL, about 100mg/mL to about 200mg/mL, about 100mg/mL to about 150mg/mL, about 125mg/mL to about 210mg/mL, about 140mg/mL to about

210mg/mL, about 100mg/mL to about 175mg/mL, about 125mg/mL to about 175mg/mL, or about 135mg/mL to about 165mg/mL. In some embodiments, the antibody concentration is any concentration within a range having an upper limit (in mg/mL) of about any of: 210, 200, 190, 180, 170, and 160; and an independently selected lower limit (in mg/mL) of about any of: 90, 100, 110, 120, 130, and 140; wherein the upper limit is greater than the lower limit. In some embodiments, the antibody is in a concentration of about any one of 90mg/mL, 100mg/mL, 110mg/mL, 120mg/mL, 130mg/mL, 140mg/mL, 150mg/mL, 160mg/mL, 170mg/mL, 180mg/mL, 190mg/mL, 200mg/mL, or 210mg/mL. In some embodiments, the antibody is in a concentration (in mg/mL) of at least about any of: 90, 100, 110, 120, 130, and 140; and optionally less than about 210mg/mL. In some embodiments, the antibody is in a concentration of about 150mg/mL.

[0079] Buffers can be used to control the pH in a range which optimizes the therapeutic effectiveness, especially if stability is pH dependent. The instant application demonstrates that certain buffers provide for stable, soluble, high-concentration formulations of certain anti-Siglec-8 antibodies, while other buffers lead to unacceptable gel formation or turbidity. In some embodiments, the formulation comprises histidine or sodium acetate.

[0080] In some embodiments, the formulation comprises histidine. In some embodiments, the formulation comprises L-histidine or a salt thereof (*e.g.*, L-histidine hydrochloride). In some embodiments, the formulation comprises L-histidine or a salt thereof (*e.g.*, L-histidine hydrochloride) at a concentration of about 10mM to about 25mM. In some embodiments, the formulation comprises L-histidine or a salt thereof (*e.g.*, L-histidine hydrochloride) at a concentration of about 10mM to about 15mM, about 10mM to about 20mM, about 10mM to about 25mM, about 15mM to about 20mM, about 15mM to about 25mM, or about any one of 10mM, 12.5mM, 15mM, 17.5mM, 20mM, 22.5mM, or 25mM. In some embodiments, the formulation comprises L-histidine or a salt thereof (*e.g.*, L-histidine hydrochloride) at a concentration of about 15mM.

[0081] In some embodiments, the formulation comprises sodium acetate. In some embodiments, the formulation comprises sodium acetate at a concentration of about 10mM to about 25mM. In some embodiments, the formulation comprises sodium acetate at a concentration of about 10mM to about 15mM, about 10mM to about 20mM, about 10mM to about 25mM, about 15mM to about 20mM, about 15mM to about 25mM, or about any one of

10mM, 12.5mM, 15mM, 17.5mM, 20mM, 22.5mM, or 25mM. In some embodiments, the formulation comprises sodium acetate at a concentration of about 15mM.

[0082] In some embodiments, the pH of the formulation is between 5.0 and 6.3. In some embodiments, the pH of the formulation is between 5.2 and 6.3, between 5.4 and 6.3, between 5.5 and 6.3, between 5.2 and 6.0, between 5.4 and 6.0, between 5.5 and 6.0, or any one of 5.0, 5.2, 5.4, 5.6, 5.8, 6.0, 6.2, or 6.3. In some embodiments, the formulation comprises histidine (*e.g.*, L-histidine or a salt thereof such as L-histidine hydrochloride), and the pH of the liquid formulation is 6.0. In some embodiments, the formulation comprises sodium acetate, and the pH of the liquid formulation is about 5.2 to about 5.8, *e.g.*, 5.5.

[0083] Additional excipients include agents which can serve as one or more of the following: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) and agents preventing denaturation or adherence to the container wall. Surprisingly, the instant application demonstrates that certain excipients are not suitable for high-concentration formulations of certain anti-Siglec-8 antibodies. For example, a salting out effect was observed in the presence of arginine at high concentration, and also observed in the presence of sodium chloride.

[0084] In some embodiments, the formulation further comprises a sugar. In some embodiments, the formulation further comprises trehalose (*e.g.*, trehalose dihydrate). In some embodiments, the formulation further comprises trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 4% to about 10%, about 5% to about 10%, about 4% to about 7.5%, about 5% to about 7.5%, about 4% to about 6%, about 5% to about 7.5%, about 6% to about 10%, about 6% to about 7.5%, or about any one of the following: 4%, 4.5%, 5%, 5.5%, 6%, 6.6%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5%, or 10%. In some embodiments, the formulation further comprises trehalose dihydrate in a concentration of about 100mM to about 300mM, about 115mM to about 290mM, about 145mM to about 290mM, about 115mM to about 220mM, about 145mM to about 220mM, about 115mM to about 175mM, about 145mM to about 220mM, about 175mM to about 300mM, about 150mM to about 220mM, about 150mM to about 200mM, or about any one of the following: 100mM, 115mM, 120mM, 125mM, 130mM, 145mM, 150mM, 165mM, 170mM, 175mM, 180mM, 190mM, 200mM, 210mM, 215mM, 220mM, 225mM, 230mM, 235mM, 240mM, 250mM, 255mM, 260mM, 270mM, 275mM, 290mM, or 300mM.

[0085] In some embodiments, the formulation further comprises sucrose. In some embodiments, the formulation further comprises sucrose in a concentration of about 4% to about

10%, about 5% to about 10%, about 4% to about 7.5%, about 5% to about 7.5%, about 4% to about 6%, about 5% to about 7.5%, about 6% to about 10%, about 6% to about 7.5%, or about any one of the following: 4%, 4.5%, 5%, 5.5%, 6%, 6.6%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5%, or 10%.

[0086] Non-ionic surfactants or detergents (also known as "wetting agents") can be present to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody. Suitable non-ionic surfactants include polysorbates (20, 40, 60, 65, 80, etc.), polyoxamers (184, 188, etc.), PLURONIC® polyols, TRITON®, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.), lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl celluose and carboxymethyl cellulose. Anionic detergents that can be used include sodium lauryl sulfate, dioctyle sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride.

[0087] In some embodiments, the formulation further comprises polysorbate 80. In some embodiments, the formulation further comprises polysorbate 80 in a concentration (w/v) of about 0.0225% to about 0.0275%, about 0.0225% to about 0.0270%, about 0.0225% to about 0.0265%, about 0.0225% to about 0.0260%, about 0.0225% to about 0.0250%, or about any one of 0.0225%, 0.0230%, 0.0235%, 0.0240%,0.0245%, 0.0250%, 0.0255%, 0.0260%, 0.0265%, 0.0270%, or 0.0275%.

[0088] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 135mg/mL to about 165mg/mL (*e.g.*, about 150mg/mL), about 10mM to about 25mM L-histidine or L-histidine hydrochloride (*e.g.*, about 15mM), trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 4% to about 10%, and polysorbate 80 in a concentration (w/v) of about 0.0225% to about 0.0275% (*e.g.*, about 0.025%), wherein the pH of the liquid formulation is between 5.0 and 6.3.

[0089] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 135mg/mL to about 165mg/mL (*e.g.*, about 150mg/mL), about 10mM to about 25mM L-histidine or L-histidine hydrochloride (*e.g.*, about 15mM), trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 150mM to about 200mM (*e.g.*, about 175mM),

and polysorbate 80 in a concentration (w/v) of about 0.0225% to about 0.0275% (*e.g.*, about 0.025%), wherein the pH of the liquid formulation is between 5.0 and 6.3.

[0090] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 135mg/mL to about 165mg/mL (*e.g.*, about 150mg/mL), about 10mM to about 25mM sodium acetate (*e.g.*, about 15mM), trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 4% to about 10%, and polysorbate 80 in a concentration (w/v) of about 0.0225% to about 0.0275% (*e.g.*, about 0.025%), wherein the pH of the liquid formulation is between 5.0 and 6.3.

[0091] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 135mg/mL to about 165mg/mL (*e.g.*, about 150mg/mL), about 10mM to about 25mM sodium acetate (*e.g.*, about 15mM), trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 150mM to about 200mM (*e.g.*, about 175mM), and polysorbate 80 in a concentration (w/v) of about 0.0225% to about 0.0275% (*e.g.*, about 0.025%), wherein the pH of the liquid formulation is between 5.0 and 6.3.

[0092] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 135mg/mL to about 165mg/mL (*e.g.*, about 150mg/mL), about 10mM to about 25mM L-histidine or L-histidine hydrochloride (*e.g.*, about 15mM), sucrose in a concentration of about 4% to about 10%, and polysorbate 80 in a concentration (w/v) of about 0.0225% to about 0.0275% (*e.g.*, about 0.025%), wherein the pH of the liquid formulation is between 5.0 and 6.3.

[0093] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 135mg/mL to about 165mg/mL (*e.g.*, about 150mg/mL), about 10mM to about 25mM sodium acetate (*e.g.*, about 15mM), sucrose in a concentration of about 4% to about 10%, and polysorbate 80 in a concentration (w/v) of about 0.0225% to about 0.0275% (*e.g.*, about 0.025%), wherein the pH of the liquid formulation is between 5.0 and 6.3.

[0094] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 135mg/mL to about 165mg/mL (*e.g.*, about 150mg/mL), about 10mM to about 25mM L-histidine or L-histidine hydrochloride (*e.g.*, about 15mM), sucrose in a concentration of about 4% to about 10%, and polysorbate 80 in a concentration (w/v) of about 0.0225% to about 0.0275% (*e.g.*, about 0.025%), wherein the pH of the liquid formulation is 6.0.

[0095] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 135mg/mL to about 165mg/mL (*e.g.*, about 150mg/mL), about 10mM to

about 25mM L-histidine or L-histidine hydrochloride (*e.g.*, about 15mM), trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 150mM to about 200mM (*e.g.*, about 175mM), and polysorbate 80 in a concentration (w/v) of about 0.0225% to about 0.0275% (*e.g.*, about 0.025%), wherein the pH of the liquid formulation is 6.0.

[0096] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 135mg/mL to about 165mg/mL (*e.g.*, about 150mg/mL), about 10mM to about 25mM sodium acetate (*e.g.*, about 15mM), trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 150mM to about 200mM (*e.g.*, about 175mM), and polysorbate 80 in a concentration (w/v) of about 0.0225% to about 0.0275% (*e.g.*, about 0.025%), wherein the pH of the liquid formulation is 6.0.

[0097] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 135mg/mL to about 165mg/mL (*e.g.*, about 150mg/mL), about 10mM to about 25mM sodium acetate (*e.g.*, about 15mM), sucrose in a concentration of about 4% to about 10%, and polysorbate 80 in a concentration (w/v) of about 0.0225% to about 0.0275% (*e.g.*, about 0.025%), wherein the pH of the liquid formulation is 5.5.

[0098] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 135mg/mL to about 165mg/mL (*e.g.*, about 150mg/mL), about 10mM to about 25mM sodium acetate (*e.g.*, about 15mM), sucrose in a concentration of about 4% to about 10%, and polysorbate 80 in a concentration (w/v) of about 0.0225% to about 0.0275% (*e.g.*, about 0.025%), wherein the pH of the liquid formulation is between 5.0 and 6.3.

[0099] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 150mg/mL, about 15mM L-histidine or L-histidine hydrochloride, trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 175mM, and polysorbate 80 in a concentration (w/v) of about 0.025%, wherein the pH of the liquid formulation is 6.0.

[0100] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 150mg/mL, about 15mM sodium acetate, trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 175mM, and polysorbate 80 in a concentration (w/v) of about 0.025%, wherein the pH of the liquid formulation is 5.5.

[0101] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 150mg/mL, about 15mM L-histidine or L-histidine hydrochloride, sucrose in a concentration of about 5%, and polysorbate 80 in a concentration (w/v) of about 0.025%, wherein the pH of the liquid formulation is 6.0.

[0102] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 150mg/mL, about 15mM sodium acetate, sucrose in a concentration of about 5%, and polysorbate 80 in a concentration (w/v) of about 0.025%, wherein the pH of the liquid formulation is 5.2. In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 150mg/mL, about 15mM sodium acetate, sucrose in a concentration of about 7.5%, and polysorbate 80 in a concentration (w/v) of about 0.025%, wherein the pH of the liquid formulation is 5.2.

[0103] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 150mg/mL, about 15mM sodium acetate, trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 5%, and polysorbate 80 in a concentration (w/v) of about 0.025%, wherein the pH of the liquid formulation is 5.2.

[0104] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 150mg/mL, about 15mM sodium acetate, trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 7.5%, and polysorbate 80 in a concentration (w/v) of about 0.025%, wherein the pH of the liquid formulation is 5.2.

[0105] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 150mg/mL, about 15mM sodium acetate, sucrose in a concentration of about 5%, and polysorbate 80 in a concentration (w/v) of about 0.025%, wherein the pH of the liquid formulation is 5.8.

[0106] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 150mg/mL, about 15mM sodium acetate, sucrose in a concentration of about 7.5%, and polysorbate 80 in a concentration (w/v) of about 0.025%, wherein the pH of the liquid formulation is 5.8.

[0107] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 150mg/mL, about 15mM sodium acetate, trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 5%, and polysorbate 80 in a concentration (w/v) of about 0.025%, wherein the pH of the liquid formulation is 5.8.

[0108] In some embodiments, the formulation comprises the anti-Siglec-8 antibody in a concentration of about 150mg/mL, about 15mM sodium acetate, trehalose (*e.g.*, trehalose dihydrate) in a concentration of about 7.5%, and polysorbate 80 in a concentration (w/v) of about 0.025%, wherein the pH of the liquid formulation is 5.8.

[0109] In order for the formulations to be used for in vivo administration, they must be sterile. The formulation may be rendered sterile by filtration through sterile filtration membranes. The therapeutic compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0110] The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, e.g., by subcutaneous injection.

[0111] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active compounds are suitably present in combination in amounts that are effective for the purpose intended.

[0112] The formulations of the present disclosure may be used to administer various doses of the antibody to an individual. In some embodiments, the antibody that binds to human Siglec-8 is administered to the individual at between 0.1mg/kg and 10mg/kg in one or more dose(s). In some embodiments, the antibody that binds to human Siglec-8 is administered to the individual at between 1mg/kg and 10mg/kg in one or more dose(s). In some embodiments, the antibody that binds to human Siglec-8 is administered to the individual at between 0.1mg/kg and 3mg/kg in one or more dose(s). In some embodiments, the antibody that binds to human Siglec-8 is administered to the individual at between 0.1mg/kg and 1mg/kg in one or more dose(s). In some embodiments, the antibody that binds to human Siglec-8 is administered to the individual at between about 1mg/kg and about 3mg/kg in one or more dose(s). In some embodiments, the antibody that binds to human Siglec-8 is administered to the individual at 0.3mg/kg, 1mg/kg, 3mg/kg, or 5mg/kg in one or more dose(s). In some embodiments, the antibody that binds to human Siglec-8 is administered to the individual at 300mg in one or more dose(s). In some embodiments, the antibody that binds to human Siglec-8 is administered to the individual at about any of 0.1 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg, 2.0 mg/kg, 2.5 mg/kg, 3.0 mg/kg, 3.5 mg/kg, 4.0 mg/kg, 4.5 mg/kg, 5.0 mg/kg, 5.5 mg/kg, 6.0 mg/kg, 6.5 mg/kg, 7.0 mg/kg, 7.5 mg/kg, 8.0 mg/kg, 8.5 mg/kg, 9.0 mg/kg, 9.5 mg/kg, or 10.0 mg/kg in one or more dose(s).

[0113] Antibodies described herein that bind to human Siglec-8 can be used either alone or in combination with other agents in the methods described herein. Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are

included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the present disclosure can occur prior to, simultaneously, and/or following, administration of the one or more additional therapeutic agents. In some embodiments, administration of an anti-Siglec-8 antibody described herein and administration of one or more additional therapeutic agents occur within about one month, about two months, about three months, about four months, about five months or about six months of each other. In some embodiments, administration of an anti-Siglec-8 antibody described herein and administration of one or more additional therapeutic agents occur within about one week, about two weeks or about three weeks of each other. In some embodiments, administration of an anti-Siglec-8 antibody described herein and administration of one or more additional therapeutic agents occur within about one day, about two days, about three days, about four days, about five days, or about six days of each other.

[0114] The formulations of the present disclosure can be administered to treat or prevent a variety of indications, *e.g.*, for treatment of eosinophil- and/or mast cell-related diseases or disorders. In some embodiments, the individual to be treated with a formulation of the present disclosure has or has been diagnosed with a disease or disorder characterized by one or more of: an increase in activated eosinophils, increased activity of mast cells expressing Siglec-8, an increase in eosinophils and/or mast cells, or increased activation of eosinophils and/or mast cells. In some embodiments, the individual to be treated with a formulation of the present disclosure has or has been diagnosed with one or more diseases or disorders selected from the group consisting of: chronic rhinosinusitis with concomitant asthma, aspirin-exacerbated respiratory disease, adult onset non-atopic asthma with sinus disease, chronic obstructive pulmonary disease, fibrotic disease, pre-fibrotic disease, advanced systemic mastocytosis, indolent systemic mastocytosis (ISM), inflammatory bowel disease (IBD), eosinophilic esophagitis (EOE), eosinophilic gastritis (EG), eosinophilic gastroenteritis (EGE), eosinophilic colitis (EOC), eosinophilic duodenitis, mast cell gastritis or mast cell gastroenteritis, gastritis or gastroenteritis with elevated mast cells, irritable bowel syndrome, irritable bowel syndrome with elevated mast cells, functional gastrointestinal disease, functional dyspepsia, allergic conjunctivitis, giant papillary conjunctivitis, chronic urticaria, allergic bronchopulmonary aspergillosis (ABPA), allergic asthma, asthma with eosinophil or mast cell phenotype, eosinophilic granulomatosis with polyangiitis (EGPA), celiac disease, gastroparesis, hypereosinophilic syndrome, atopic dermatitis, anaphylaxis, angioedema, mast cell activation syndrome/disorder, and eosinophilic

fasciitis. In some embodiments, the individual to be treated with a formulation of the present disclosure is a human. In some embodiments, prior to administration of the composition, the individual has failed a prior treatment (*e.g.,* a standard of care treatment) for one or more of the indications described above. In some embodiments, prior to administration of the composition, the individual has had disease symptoms not adequately controlled by a prior treatment (*e.g.,* a standard of care treatment) for one or more of the indications described above.

[0115] In some embodiments according to any of the embodiments described herein, the administration results in a decrease in eosinophil count as compared to eosinophil count prior to administration of the composition, *e.g.*, in a biopsy sample obtained from the individual (for tissue eosinophil count) or peripheral blood (for blood eosinophil count). In some embodiments according to any of the embodiments described herein, the administration results in a decrease in mast cell activation as compared to mast cell activation prior to administration of the composition, *e.g.*, in a biopsy sample obtained from tissue or peripheral blood. In some embodiments according to any of the embodiments described herein, the administration results in a decrease in one or more symptoms in the individual, as compared to one or more symptoms in the individual prior to administration of the composition. In some embodiments, the administration (*e.g.,* subcutaneous injection) leads to a decrease in eosinophil count comparable to intravenous infusion, *e.g.*, in a biopsy sample obtained from the individual (for tissue eosinophil count) or peripheral blood (for blood eosinophil count). In some embodiments, the administration (*e.g.,* subcutaneous injection) results in a blood eosinophil count of less than 10^3/mL, or an undetectable level, 1 hour, 3 hours, 15 days, 35 days, 56 days, and/or 85 days after administration.

Antibodies

[0116] Certain aspects of the present disclosure provide isolated antibodies that bind to a human Siglec-8 (*e.g.*, an agonist antibody that binds to human Siglec-8). In some embodiments, an anti-Siglec-8 antibody described herein has one or more of the following characteristics: (1) binds a human Siglec-8; (2) binds to an extracellular domain of a human Siglec-8; (3) binds a human Siglec-8 with a higher affinity than mouse antibody 2E2 and/or mouse antibody 2C4; (4) binds a human Siglec-8 with a higher avidity than mouse antibody 2E2 and/or mouse antibody 2C4; (5) has a T_m of about 70°C-72°C or higher in a thermal shift assay; (6) has a reduced degree of fucosylation or is non-fucosylated; (7) binds a human Siglec-8 expressed on eosinophils and

induces apoptosis of eosinophils; (8) binds a human Siglec-8 expressed on mast cells and depletes or reduces the number of mast cells; (9) binds a human Siglec-8 expressed on mast cells and inhibits FcεRI-dependent activities of mast cells (*e.g.*, histamine release, PGD_2 release, Ca^{2+} flux, and/or β-hexosaminidase release, etc.); (10) has been engineered to improve ADCC activity; (11) binds a human Siglec-8 expressed on mast cells and kills mast cells by ADCC activity (*in vitro*, and/or *in vivo*); (12) binds to Siglec-8 of a human and a non-human primate; (13) binds to Domain 1, Domain 2, and/or Domain 3 of human Siglec-8, or binds a Siglec-8 polypeptide comprising Domain 1, Domain 2, and/or Domain 3 of human Siglec-8 (e.g., fusion proteins described herein); and (14) depletes activated eosinophils with an EC_{50} less than the EC_{50} of mouse antibody 2E2 or 2C4. Any of the antibodies described in U.S. Pat. No. 9,546,215 and/or WO2015089117 may find use in the methods, compositions, and kits provided herein.

[0117] In one aspect, the present disclosure provides antibodies that bind to a human Siglec-8. In some embodiments, the human Siglec-8 comprises an amino acid sequence of SEQ ID NO:72. In some embodiments, the human Siglec-8 comprises an amino acid sequence of SEQ ID NO:73. In some embodiments, an antibody described herein binds to a human Siglec-8 expressed on mast cells and depletes or reduces the number of mast cells. In some embodiments, an antibody described herein binds to a human Siglec-8 expressed on mast cells and inhibits mast cell-mediated activity.

[0118] In one aspect, the invention provides antibodies that bind to a human Siglec-8. In some embodiments, the human Siglec-8 comprises an amino acid sequence of SEQ ID NO:72. In some embodiments, the human Siglec-8 comprises an amino acid sequence of SEQ ID NO:73. In some embodiments, the antibody described herein binds to an epitope in Domain 1 of human Siglec-8, wherein Domain 1 comprises the amino acid sequence of SEQ ID NO: 112. In some embodiments, the antibody described herein binds to an epitope in Domain 2 of human Siglec-8, wherein Domain 2 comprises the amino acid sequence of SEQ ID NO: 113. In some embodiments, the antibody described herein binds to an epitope in Domain 3 of human Siglec-8, wherein Domain 3 comprises the amino acid sequence of SEQ ID NO: 114. In some embodiments, the antibody described herein binds to a fusion protein comprising the amino acid of SEQ ID NO:116 but not to a fusion protein comprising the amino acid of SEQ ID NO:115. In some embodiments, the antibody described herein binds to a fusion protein comprising the amino acid of SEQ ID NO:117 but not to a fusion protein comprising the amino acid of SEQ ID NO:115. In some embodiments, the antibody described herein binds to a fusion protein

comprising the amino acid of SEQ ID NO:117 but not to a fusion protein comprising the amino acid of SEQ ID NO:116. In some embodiments, the antibody described herein binds to a linear epitope in the extracellular domain of human Siglec-8. In some embodiments, the antibody described herein binds to a conformational epitope in the extracellular domain of human Siglec-8. In some embodiments, an antibody described herein binds to a human Siglec-8 expressed on eosinophils and induces apoptosis of eosinophils. In some embodiments, an antibody described herein binds to a human Siglec-8 expressed on mast cells and depletes mast cells. In some embodiments, an antibody described herein binds to a human Siglec-8 expressed on mast cells and inhibits mast cell-mediated activity. In some embodiments, an antibody described herein binds to a human Siglec-8 expressed on mast cells and kills mast cells by ADCC activity. In some embodiments, an antibody described herein depletes mast cells and inhibits mast cell activation. In some embodiments, an antibody herein depletes activated eosinophils and inhibits mast cell activation. In some embodiments, an antibody herein (*e.g.*, a non-fucosylated anti-Siglec-8 antibody) depletes blood eosinophils and inhibits mast cell activation. In some embodiments, an antibody herein (*e.g.*, a non-fucosylated anti-Siglec-8 antibody) depletes eosinophils from the peripheral blood and inhibits mast cell activation.

[0119] Provided herein is an isolated anti-Siglec-8 antibody that binds to human Siglec-8 and non-human primate Siglec-8. Identification of antibodies with primate cross-reactivity would be useful for preclinical testing of anti-Siglec-8 antibodies in non-human primates. In one aspect, the invention provides antibodies that bind to a non-human primate Siglec-8. In one aspect, the invention provides antibodies that bind to a human Siglec-8 and a non-human primate Siglec-8. In some embodiments, the non-human primate Siglec-8 comprises an amino acid sequence of SEQ ID NO:118 or a portion thereof. In some embodiments, the non-human primate Siglec-8 comprises an amino acid sequence of SEQ ID NO:119 or a portion thereof. In some embodiments, the non-human primate is a baboon (*e.g.*, *Papio Anubis*). In some embodiments, the antibody that binds to a human Siglec-8 and a non-human primate Siglec-8, binds to an epitope in Domain 1 of human Siglec-8. In a further embodiment, Domain 1 of human Siglec-8 comprises the amino acid sequence of SEQ ID NO:112. In some embodiments, the antibody that binds to a human Siglec-8 and a non-human primate Siglec-8, binds to an epitope in Domain 3 of human Siglec-8. In a further embodiment, Domain 3 of human Siglec-8 comprises the amino acid sequence of SEQ ID NO:114. In some embodiments, the antibody that binds to a human Siglec-8 and a non-human primate Siglec-8 is a humanized antibody, a chimeric

antibody, or a human antibody. In some embodiments, the antibody that binds to a human Siglec-8 and a non-human primate Siglec-8 is a murine antibody. In some embodiments, the antibody that binds to a human Siglec-8 and a non-human primate Siglec-8 is a human IgG1 antibody.

[0120] In one aspect, an anti-Siglec-8 antibody described herein is a monoclonal antibody. In one aspect, an anti-Siglec-8 antibody described herein is an antibody fragment (including antigen-binding fragment), *e.g.*, a Fab, Fab′-SH, Fv, scFv, or $(Fab')_2$ fragment. In one aspect, an anti-Siglec-8 antibody described herein comprises an antibody fragment (including antigen-binding fragment), *e.g.*, a Fab, Fab′-SH, Fv, scFv, or $(Fab')_2$ fragment. In one aspect, an anti-Siglec-8 antibody described herein is a chimeric, humanized, or human antibody. In one aspect, any of the anti-Siglec-8 antibodies described herein are purified.

[0121] In one aspect, anti-Siglec-8 antibodies that compete with murine 2E2 antibody and murine 2C4 antibody binding to Siglec-8 are provided. Anti-Siglec-8 antibodies that bind to the same epitope as murine 2E2 antibody and murine 2C4 antibody are also provided. Murine antibodies to Siglec-8, 2E2 and 2C4 antibody are described in U.S. Pat. No. 8,207,305; U.S. Pat. No. 8,197,811, U.S. Pat. No. 7,871,612, and U.S. Pat. No. 7,557,191.

[0122] In one aspect, anti-Siglec-8 antibodies that compete with any anti-Siglec-8 antibody described herein (*e.g.*, HEKA, HEKF, 1C3, 1H10, 4F11, 2C4, 2E2) for binding to Siglec-8 are provided. Anti-Siglec-8 antibodies that bind to the same epitope as any anti-Siglec-8 antibody described herein (*e.g.*, HEKA, HEKF, 1C3, 1H10, 4F11, 2C4, 2E2) are also provided.

[0123] In one aspect of the present disclosure, polynucleotides encoding anti-Siglec-8 antibodies are provided. In certain embodiments, vectors comprising polynucleotides encoding anti-Siglec-8 antibodies are provided. In certain embodiments, host cells comprising such vectors are provided. In another aspect of the present disclosure, compositions comprising anti-Siglec-8 antibodies or polynucleotides encoding anti-Siglec-8 antibodies are provided. In certain embodiments, a composition of the present disclosure is a pharmaceutical formulation for the treatment of an eosinophil- or mast cell-related disease or disorder of the present disclosure.

[0124] In one aspect, provided herein is an anti-Siglec-8 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 2C4. In one aspect, provided herein is an anti-Siglec-8 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 2E2. In some embodiments, the HVR is a Kabat CDR or a Chothia CDR.

[0125] In one aspect, provided herein is an anti-Siglec-8 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 1C3. In one aspect, provided herein is an anti-Siglec-8 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 4F11. In one aspect, provided herein is an anti-Siglec-8 antibody comprising 1, 2, 3, 4, 5, or 6 of the HVR sequences of the murine antibody 1H10. In some embodiments, the HVR is a Kabat CDR or a Chothia CDR.

[0126] In some embodiments, the antibody described herein binds to an epitope in Domain 1 of human Siglec-8, wherein Domain 1 comprises the amino acid sequence of SEQ ID NO:112. In some embodiments, the antibody described herein binds to an epitope in Domain 2 of human Siglec-8, wherein Domain 2 comprises the amino acid sequence of SEQ ID NO:113. In some embodiments, the antibody described herein binds to an epitope in Domain 3 of human Siglec-8, wherein Domain 3 comprises the amino acid sequence of SEQ ID NO:114.

[0127] In some embodiments, the antibody described herein binds to a fusion protein comprising the amino acid of SEQ ID NO:116 but not to a fusion protein comprising the amino acid of SEQ ID NO:115. In some embodiments, the antibody described herein binds to a fusion protein comprising the amino acid of SEQ ID NO:117 but not to a fusion protein comprising the amino acid of SEQ ID NO:115. In some embodiments, the antibody described herein binds to a fusion protein comprising the amino acid of SEQ ID NO:117 but not to a fusion protein comprising the amino acid of SEQ ID NO:116.

[0128] In another aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:88, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:91, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:94; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:97, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:100, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:103. In some embodiments, the antibody described herein binds to an epitope in Domain 2 of human Siglec-8, wherein Domain 2 comprises the amino acid sequence of SEQ ID NO: 113.

[0129] In another aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:89, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:92, and (iii) HVR-H3 comprising the amino

acid sequence of SEQ ID NO:95; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:98, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:101, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:104. In some embodiments, the antibody described herein binds to an epitope in Domain 3 of human Siglec-8, wherein Domain 3 comprises the amino acid sequence of SEQ ID NO: 114. In some embodiments, the antibody described herein binds to human Siglec-8 and non-human primate Siglec-8.

[0130] In another aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:90, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:93, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:96; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:99, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:102, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:105. In some embodiments, the antibody described herein binds to an epitope in Domain 1 of human Siglec-8, wherein Domain 1 comprises the amino acid sequence of SEQ ID NO: 112. In some embodiments, the antibody described herein binds to human Siglec-8 and non-human primate Siglec-8.

[0131] In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66.

[0132] In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence selected from SEQ ID NOs:67-70; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2

comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:66.

[0133] In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:71.

[0134] In another aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:61, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:62, and (iii) HVR-H3 comprising the amino acid sequence selected from SEQ ID NOs:67-70; and/or wherein the light chain variable region comprises (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:64, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:65, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:71.

[0135] In another aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:88, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:91, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:94; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:97, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:100, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:103.

[0136] In another aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:89, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:92, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:95; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:98, (ii) HVR-L2 comprising the amino acid

sequence of SEQ ID NO:101, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:104.

[0137] In another aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:90, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:93, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:96; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:99, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:102, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:105.

[0138] An anti-Siglec-8 antibody described herein may comprise any suitable framework variable domain sequence, provided that the antibody retains the ability to bind human Siglec-8. As used herein, heavy chain framework regions are designated "HC-FR1-FR4," and light chain framework regions are designated "LC-FR1-FR4." In some embodiments, the anti-Siglec-8 antibody comprises a heavy chain variable domain framework sequence of SEQ ID NO:26, 34, 38, and 45 (HC-FR1, HC-FR2, HC-FR3, and HC-FR4, respectively). In some embodiments, the anti-Siglec-8 antibody comprises a light chain variable domain framework sequence of SEQ ID NO:48, 51, 55, and 60 (LC-FR1, LC-FR2, LC-FR3, and LC-FR4, respectively). In some embodiments, the anti-Siglec-8 antibody comprises a light chain variable domain framework sequence of SEQ ID NO:48, 51, 58, and 60 (LC-FR1, LC-FR2, LC-FR3, and LC-FR4, respectively).

[0139] In one embodiment, an anti-Siglec-8 antibody comprises a heavy chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the HC-FR1-HC-FR4 sequences SEQ ID NOs:26-29 (HC-FR1), SEQ ID NOs:31-36 (HC-FR2), SEQ ID NOs:38-43 (HC-FR3), and SEQ ID NOs:45 or 46 (HC-FR4), respectively; the HVR-H1 comprises the amino acid sequence of SEQ ID NO:61; the HVR-H2 comprises the amino acid sequence of SEQ ID NO:62; and the HVR-H3 comprises an amino acid sequence of SEQ ID NO:63. In one embodiment, an anti-Siglec-8 antibody comprises a heavy chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the HC-FR1-HC-FR4 sequences SEQ ID NOs:26-29 (HC-FR1), SEQ ID NOs:31-36 (HC-FR2), SEQ ID NOs:38-43 (HC-FR3), and SEQ ID NOs:45 or 46 (HC-FR4), respectively; the HVR-H1 comprises the amino acid sequence of SEQ

ID NO:61; the HVR-H2 comprises the amino acid sequence of SEQ ID NO:62; and the HVR-H3 comprises an amino acid sequence selected from SEQ ID NOs:67-70. In one embodiment, an anti-Siglec-8 antibody comprises a light chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the LC-FR1-LC-FR4 sequences SEQ ID NOs:48 or 49 (LC-FR1), SEQ ID NOs:51-53 (LC-FR2), SEQ ID NOs:55-58 (LC-FR3), and SEQ ID NO:60 (LC-FR4), respectively; the HVR-L1 comprises the amino acid sequence of SEQ ID NO:64; the HVR-L2 comprises the amino acid sequence of SEQ ID NO:65; and the HVR-L3 comprises an amino acid sequence of SEQ ID NO:66. In one embodiment, an anti-Siglec-8 antibody comprises a light chain variable domain comprising a framework sequence and hypervariable regions, wherein the framework sequence comprises the LC-FR1-LC-FR4 sequences SEQ ID NOs:48 or 49 (LC-FR1), SEQ ID NOs:51-53 (LC-FR2), SEQ ID NOs:55-58 (LC-FR3), and SEQ ID NO:60 (LC-FR4), respectively; the HVR-L1 comprises the amino acid sequence of SEQ ID NO:64; the HVR-L2 comprises the amino acid sequence of SEQ ID NO:65; and the HVR-L3 comprises an amino acid sequence of SEQ ID NO:71. In one embodiment of these antibodies, the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:2-10 and the light chain variable domain comprises and amino acid sequence selected from SEQ ID NOs:16-22. In one embodiment of these antibodies, the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:2-10 and the light chain variable domain comprises and amino acid sequence selected from SEQ ID NOs:23 or 24. In one embodiment of these antibodies, the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:11-14 and the light chain variable domain comprises and amino acid sequence selected from SEQ ID NOs:16-22. In one embodiment of these antibodies, the heavy chain variable domain comprises an amino acid sequence selected from SEQ ID NOs:11-14 and the light chain variable domain comprises and amino acid sequence selected from SEQ ID NOs:23 or 24. In one embodiment of these antibodies, the heavy chain variable domain comprises an amino acid sequence of SEQ ID NO:6 and the light chain variable domain comprises and amino acid sequence of SEQ ID NO:16. In one embodiment of these antibodies, the heavy chain variable domain comprises an amino acid sequence of SEQ ID NO:6 and the light chain variable domain comprises and amino acid sequence of SEQ ID NO:21.

[0140] In some embodiments, the heavy chain HVR sequences comprise the following:

a) HVR-H1 (IYGAH (SEQ ID NO:61));

b) HVR-H2 (VIWAGGSTNYNSALMS (SEQ ID NO:62)); and

c) HVR-H3 (DGSSPYYYSMEY (SEQ ID NO:63); DGSSPYYYGMEY (SEQ ID NO:67); DGSSPYYYSMDY (SEQ ID NO:68); DGSSPYYYSMEV (SEQ ID NO:69); or DGSSPYYYGMDV (SEQ ID NO:70)).

[0141] In some embodiments, the heavy chain HVR sequences comprise the following:

a) HVR-H1 (SYAMS (SEQ ID NO:88); DYYMY (SEQ ID NO:89); or SSWMN (SEQ ID NO:90));

b) HVR-H2 (IISSGGSYTYYSDSVKG (SEQ ID NO:91); RIAPEDGDTEYAPKFQG (SEQ ID NO:92); or QIYPGDDYTNYNGKFKG (SEQ ID NO:93)); and c) HVR-H3 (HETAQAAWFAY (SEQ ID NO:94); EGNYYGSSILDY (SEQ ID NO:95); or LGPYGPFAD (SEQ ID NO:96)).

[0142] In some embodiments, the heavy chain FR sequences comprise the following:

a) HC-FR1 (EVQLVESGGGLVQPGGSLRLSCAASGFSLT (SEQ ID NO:26); EVQLVESGGGLVQPGGSLRLSCAVSGFSLT (SEQ ID NO:27); QVQLQESGPGLVKPSETLSLTCTVSGGSIS (SEQ ID NO:28); or QVQLQESGPGLVKPSETLSLTCTVSGFSLT (SEQ ID NO:29));

b) HC-FR2 (WVRQAPGKGLEWVS (SEQ ID NO:31); WVRQAPGKGLEWLG (SEQ ID NO:32); WVRQAPGKGLEWLS (SEQ ID NO: 33); WVRQAPGKGLEWVG (SEQ ID NO:34); WIRQPPGKGLEWIG (SEQ ID NO:35); or WVRQPPGKGLEWLG (SEQ ID NO:36));

c) HC-FR3 (RFTISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:38); RLSISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:39); RLTISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:40); RFSISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:41); RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR (SEQ ID NO:42); or RLSISKDNSKNQVSLKLSSVTAADTAVYYCAR (SEQ ID NO:43)); and

d) HC-FR4 (WGQGTTVTVSS (SEQ ID NO:45); or WGQGTLVTVSS (SEQ ID NO:46)).

[0143] In some embodiments, the light chain HVR sequences comprise the following:

a) HVR-L1 (SATSSVSYMH (SEQ ID NO:64));

b) HVR-L2 (STSNLAS (SEQ ID NO:65)); and

c) HVR-L3 (QQRSSYPFT (SEQ ID NO:66); or QQRSSYPYT (SEQ ID NO:71)).

[0144] In some embodiments, the light chain HVR sequences comprise the following:

a) HVR-L1 (SASSSVSYMH (SEQ ID NO:97); RASQDITNYLN (SEQ ID NO:98); or SASSSVSYMY (SEQ ID NO:99));

b) HVR-L2 (DTSKLAY (SEQ ID NO:100); FTSRLHS (SEQ ID NO:101); or DTSSLAS (SEQ ID NO:102)); and

c) HVR-L3 (QQWSSNPPT (SEQ ID NO:103); QQGNTLPWT (SEQ ID NO:104); or QQWNSDPYT (SEQ ID NO:105)).

[0145] In some embodiments, the antibody comprises:

a heavy chain variable region comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:88, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:91, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:94; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:97, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:100, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:103;

a heavy chain variable region comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:89, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:92, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:95; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:98, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:101, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:104; or

a heavy chain variable region comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO:90, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO:93, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO:96; and/or a light chain variable region comprising (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO:99, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO:102, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO:105.

[0146] In some embodiments, the light chain FR sequences comprise the following:

a) LC-FR1 (EIVLTQSPATLSLSPGERATLSC (SEQ ID NO:48); or EIILTQSPATLSLSPGERATLSC (SEQ ID NO:49));

b) LC-FR2 (WFQQKPGQAPRLLIY (SEQ ID NO:51); WFQQKPGQAPRLWIY (SEQ ID NO:52); or WYQQKPGQAPRLLIY (SEQ ID NO: 53));

c) LC-FR3 (GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC (SEQ ID NO:55);
GVPARFSGSGSGTDYTLTISSLEPEDFAVYYC (SEQ ID NO:56);
GVPARFSGSGSGTDFTLTISSLEPEDFAVYYC (SEQ ID NO:57); or
GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC (SEQ ID NO:58)); and
d) LC-FR4 (FGPGTKLDIK (SEQ ID NO:60)).

[0147] In some embodiments, provided herein is an anti-Siglec-8 antibody (*e.g.*, a humanized anti-Siglec-8) antibody that binds to human Siglec-8, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the antibody comprises:

(a) heavy chain variable domain comprising:
(1) an HC-FR1 comprising the amino acid sequence selected from SEQ ID NOs:26-29;
(2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61;
(3) an HC-FR2 comprising the amino acid sequence selected from SEQ ID NOs:31-36;
(4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62;
(5) an HC-FR3 comprising the amino acid sequence selected from SEQ ID NOs:38-43;
(6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and
(7) an HC-FR4 comprising the amino acid sequence selected from SEQ ID NOs:45-46, and/or
(b) a light chain variable domain comprising:
(1) an LC-FR1 comprising the amino acid sequence selected from SEQ ID NOs:48-49;
(2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64;
(3) an LC-FR2 comprising the amino acid sequence selected from SEQ ID NOs:51-53;
(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65;
(5) an LC-FR3 comprising the amino acid sequence selected from SEQ ID NOs:55-58;
(6) an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66; and
(7) an LC-FR4 comprising the amino acid sequence of SEQ ID NO:60.

[0148] In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain selected from SEQ ID NOs:2-10 and/or comprising a light chain variable domain selected from SEQ ID NOs:16-22. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain selected from SEQ ID NOs:2-14 and/or comprising a light chain variable domain selected from SEQ ID NOs:16-24. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain selected from SEQ ID NOs:2-10 and/or comprising a light chain variable domain selected from SEQ ID

NO:23 or 24. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain selected from SEQ ID NOs:11-14 and/or comprising a light chain variable domain selected from SEQ ID NOs:16-22. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain selected from SEQ ID NOs:11-14 and/or comprising a light chain variable domain selected from SEQ ID NO:23 or 24. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain of SEQ ID NO:6 and/or comprising a light chain variable domain selected from SEQ ID NO:16 or 21.

[0149] In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain selected from SEQ ID NOs:106-108 and/or comprising a light chain variable domain selected from SEQ ID NOs:109-111. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain of SEQ ID NO:106 and/or comprising a light chain variable domain of SEQ ID NO:109. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain of SEQ ID NO:107 and/or comprising a light chain variable domain of SEQ ID NO:110. In one aspect, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain of SEQ ID NO:108 and/or comprising a light chain variable domain of SEQ ID NO:111.

[0150] In some embodiments, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NOs:2-14. In some embodiments, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NOs:106-108. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, insertions, or deletions relative to the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to human Siglec-8. In some embodiments, the substitutions, insertions, or deletions (e.g., 1, 2, 3, 4, or 5 amino acids) occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-Siglec-8 antibody comprises a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO:6. In some embodiments, an anti-Siglec-8 antibody comprises a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NOs:106-108.

[0151] In some embodiments, provided herein is an anti-Siglec-8 antibody comprising a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NOs:16-24. In some embodiments, provided herein is an anti-Siglec-8 antibody comprising a light chain variable domain comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NOs:109-111. In some embodiments, an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity contains substitutions, insertions, or deletions relative to the reference sequence, but an antibody comprising that amino acid sequence retains the ability to bind to human Siglec-8. In some embodiments, the substitutions, insertions, or deletions (e.g., 1, 2, 3, 4, or 5 amino acids) occur in regions outside the HVRs (i.e., in the FRs). In some embodiments, an anti-Siglec-8 antibody comprises a light chain variable domain comprising an amino acid sequence of SEQ ID NO:16 or 21. In some embodiments, an anti-Siglec-8 antibody comprises a heavy chain variable domain comprising an amino acid sequence selected from SEQ ID NOs:109-111.

[0152] In one aspect, the present disclosure provides an anti-Siglec-8 antibody comprising (a) one, two, or three VH HVRs selected from those shown in Table 1 and/or (b) one, two, or three VL HVRs selected from those shown in Table 1.

[0153] In one aspect, the present disclosure provides an anti-Siglec-8 antibody comprising (a) one, two, or three VH HVRs selected from those shown in Table 2 and/or (b) one, two, or three VL HVRs selected from those shown in Table 2.

[0154] In one aspect, the present disclosure provides an anti-Siglec-8 antibody comprising (a) one, two, three or four VH FRs selected from those shown in Table 3 and/or (b) one, two, three or four VL FRs selected from those shown in Table 3.

[0155] In some embodiments, provided herein is an anti-Siglec-8 antibody comprising a heavy chain variable domain and/or a light chain variable domain of an antibody shown in Table 4, for example, HAKA antibody, HAKB antibody, HAKC antibody, etc.

Table 1. Amino acid sequences of HVRs of antibodies

Antibody Chain	**HVR1**	**HVR2**	**HVR3**
2E2 antibody			
Heavy chain	IYGAH	VIWAGGSTNYNSALMS	DGSSPYYYSMEY

	SEQ ID NO:61	SEQ ID NO:62	SEQ ID NO:63
Light chain	SATSSVSYMH SEQ ID NO:64	STSNLAS SEQ ID NO:65	QQRSSYPFT SEQ ID NO:66
Humanized Heavy Chain Variants 2E2 RHA, 2E2 RHB, 2E2 RHC, 2E2 RHD, 2E2 RHE, 2E2 RHF, 2E2 RHG, 2E2 RHA2, and 2E2 RHB2			
Heavy chain	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYSMEY SEQ ID NO:63
Humanized Light Chain Variants 2E2 RKA, 2E2 RKB, 2E2 RKC, 2E2 RKD, 2E2 RKE, 2E2 RKF, and 2E2 RKG			
Light chain	SATSSVSYMH SEQ ID NO:64	STSNLAS SEQ ID NO:65	QQRSSYPFT SEQ ID NO:66
Humanized Heavy Chain Variants 2E2 RHE S-G, 2E2 RHE E-D, 2E2 RHE Y-V, and 2E2 RHE triple			
2E2 RHE S-G	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYGMEY SEQ ID NO:67
2E2 RHE E-D	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYSMDY SEQ ID NO:68
2E2 RHE Y-V	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYSMEV SEQ ID NO:69
2E2 RHE triple	IYGAH SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYGMDV SEQ ID NO:70
Humanized Light Chain Variants 2E2 RKA F-Y and 2E2 RKF F-Y			
2E2 RKA F-Y	SATSSVSYMH SEQ ID NO:64	STSNLAS SEQ ID NO:65	QQRSSYPYT SEQ ID NO:71
2E2 RKF F-Y	SATSSVSYMH SEQ ID NO:64	STSNLAS SEQ ID NO:65	QQRSSYPYT SEQ ID NO:71

Table 2. Amino acid sequences of HVRs from murine 1C3, 1H10, and 4F11 antibodies

Antibody	Chain	HVR1	HVR2	HVR3
1C3	Heavy Chain	SYAMS SEQ ID NO:88	IISSGGSYTYYSDSVKG SEQ ID NO:91	HETAQAAWFAY SEQ ID NO:94
1H10	Heavy Chain	DYYMY SEQ ID NO:89	RIAPEDGDTEYAPKFQG SEQ ID NO:92	EGNYYGSSILDY SEQ ID NO:95
4F11	Heavy Chain	SSWMN SEQ ID NO:90	QIYPGDDYTNYNGKFKG SEQ ID NO:93	LGPYGPFAD SEQ ID NO:96
1C3	Light Chain	SASSSVSYMH SEQ ID NO:97	DTSKLAY SEQ ID NO:100	QQWSSNPPT SEQ ID NO:103
1H10	Light Chain	RASQDITNYLN SEQ ID NO:98	FTSRLHS SEQ ID NO:101	QQGNTLPWT SEQ ID NO:104

4F11	Light Chain	SASSSVSYMY SEQ ID NO:99	DTSSLAS SEQ ID NO:102	QQWNSDPYT SEQ ID NO:105

Table 3. Amino acid sequences of FRs of antibodies

Heavy Chain	**FR1**	**FR2**	**FR3**	**FR4**
2E2	QVQLKESGPGLVA PSQSLSITCTVSGFS LT (SEQ ID NO:25)	WVRQPPGKGLEW LG (SEQ ID NO:30)	RLSISKDNSKSQVF LKINSLQTDDTAL YYCAR (SEQ ID NO:37)	WGQGTSVTVSS (SEQ ID NO:44)
2E2 RHA	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VS (SEQ ID NO:31)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHB	EVQLVESGGGLVQ PGGSLRLSCAVSGF SLT (SEQ ID NO:27)	WVRQAPGKGLEW LG (SEQ ID NO:32)	RLSISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:39)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHC	EVQLVESGGGLVQ PGGSLRLSCAVSGF SLT (SEQ ID NO:27)	WVRQAPGKGLEW VS (SEQ ID NO:31)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHD	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW LS (SEQ ID NO:33)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHE	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VG (SEQ ID NO:34)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHF	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VS (SEQ ID NO:31)	RLTISKDNSKNTV YLQMNSLRAEDTA VYYCAR (SEQ ID NO:40)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHG	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT (SEQ ID NO:26)	WVRQAPGKGLEW VS (SEQ ID NO:31)	RFSISKDNSKNTVY LQMNSLRAEDTAV YYCAR (SEQ ID NO:41)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHA2	QVQLQESGPGLVK	WIRQPPGKGLEWI	RVTISVDTSKNQFS	WGQGTLVTVSS

	PSETLSLTCTVSGGSIS (SEQ ID NO:28)	G (SEQ ID NO:35)	LKLSSVTAADTAVYYCAR (SEQ ID NO:42)	(SEQ ID NO:46)
2E2 RHB2	QVQLQESGPGLVKPSETLSLTCTVSGFSLT (SEQ ID NO:29)	WVRQPPGKGLEWLG (SEQ ID NO:36)	RLSISKDNSKNQVSLKLSSVTAADTAVYYCAR (SEQ ID NO:43)	WGQGTLVTVSS (SEQ ID NO:46)
2E2 RHE S-G	EVQLVESGGGLVQPGGSLRLSCAASGFSLT (SEQ ID NO:26)	WVRQAPGKGLEWVG (SEQ ID NO:34)	RFTISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHE E-D	EVQLVESGGGLVQPGGSLRLSCAASGFSLT (SEQ ID NO:26)	WVRQAPGKGLEWVG (SEQ ID NO:34)	RFTISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHE Y-V	EVQLVESGGGLVQPGGSLRLSCAASGFSLT (SEQ ID NO:26)	WVRQAPGKGLEWVG (SEQ ID NO:34)	RFTISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
2E2 RHE triple	EVQLVESGGGLVQPGGSLRLSCAASGFSLT (SEQ ID NO:26)	WVRQAPGKGLEWVG (SEQ ID NO:34)	RFTISKDNSKNTVYLQMNSLRAEDTAVYYCAR (SEQ ID NO:38)	WGQGTTVTVSS (SEQ ID NO:45)
Light Chain	**FR1**	**FR2**	**FR3**	**FR4**
2E2	QIILTQSPAIMSASPGEKVSITC (SEQ ID NO:47)	WFQQKPGTSPKLWIY (SEQ ID NO:50)	GVPVRFSGSGSGTSYSLTISRMEAEDAATYYC (SEQ ID NO:54)	FGSGTKLEIK (SEQ ID NO:59)
RKA	EIVLTQSPATLSLSPGERATLSC (SEQ ID NO:48)	WFQQKPGQAPRLLIY (SEQ ID NO:51)	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKB	EIILTQSPATLSLSPGERATLSC (SEQ ID NO:49)	WFQQKPGQAPRLWIY (SEQ ID NO:52)	GVPARFSGSGSGTDYTLTISSLEPEDFAVYYC (SEQ ID NO:56)	FGPGTKLDIK (SEQ ID NO:60)
RKC	EIILTQSPATLSLSPGERATLSC	WFQQKPGQAPRLLIY	GIPARFSGSGSGTDFTLTISSLEPEDFAV	FGPGTKLDIK (SEQ ID NO:60)

	(SEQ ID NO:49)	(SEQ ID NO:51)	YYC (SEQ ID NO:55)	
RKD	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRL WIY (SEQ ID NO:52)	GIPARFSGSGSGTD FTLTISSLEPEDFAV YYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKE	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRLL IY (SEQ ID NO:51)	GVPARFSGSGSGT DFTLTISSLEPEDFA VYYC (SEQ ID NO:57)	FGPGTKLDIK (SEQ ID NO:60)
RKF	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRLL IY (SEQ ID NO:51)	GIPARFSGSGSGTD YTLTISSLEPEDFA VYYC (SEQ ID NO:58)	FGPGTKLDIK (SEQ ID NO:60)
RKG	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WYQQKPGQAPRL LIY (SEQ ID NO:53)	GIPARFSGSGSGTD FTLTISSLEPEDFAV YYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKA F-Y	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRLL IY (SEQ ID NO:51)	GIPARFSGSGSGTD FTLTISSLEPEDFAV YYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKF F-Y	EIVLTQSPATLSLSP GERATLSC (SEQ ID NO:48)	WFQQKPGQAPRLL IY (SEQ ID NO:51)	GIPARFSGSGSGTD YTLTISSLEPEDFA VYYC (SEQ ID NO:58)	FGPGTKLDIK (SEQ ID NO:60)

Table 4. Amino acid sequences of variable regions of antibodies

Antibody Name	Variable Heavy Chain	Variable Light Chain
ch2C4	ch2C4 VH	ch2C4 VK
ch2E2	ch2E2 VH (SEQ ID NO:1)	ch2E2 VK (SEQ ID NO:15)
cVHKA	ch2E2 VH (SEQ ID NO:1)	2E2 RKA (SEQ ID NO:16)
cVHKB	ch2E2 VH (SEQ ID NO:1)	2E2 RKB (SEQ ID NO:17)
HAcVK	2E2 RHA (SEQ ID NO:2)	ch2E2 VK (SEQ ID NO:15)

HBcVK	2E2 RHB (SEQ ID NO:3)	ch2E2 VK (SEQ ID NO:15)
HAKA	2E2 RHA (SEQ ID NO:2)	2E2 RKA (SEQ ID NO:16)
HAKB	2E2 RHA (SEQ ID NO:2)	2E2 RKB (SEQ ID NO:17)
HAKC	2E2 RHA (SEQ ID NO:2)	2E2 RKC (SEQ ID NO:18)
HAKD	2E2 RHA (SEQ ID NO:2)	2E2 RKD (SEQ ID NO:19)
HAKE	2E2 RHA (SEQ ID NO:2)	2E2 RKE (SEQ ID NO:20)
HAKF	2E2 RHA (SEQ ID NO:2)	2E2 RKF (SEQ ID NO:21)
HAKG	2E2 RHA (SEQ ID NO:2)	2E2 RKG (SEQ ID NO:22)
HBKA	2E2 RHB (SEQ ID NO:3)	2E2 RKA (SEQ ID NO:16)
HBKB	2E2 RHB (SEQ ID NO:3)	2E2 RKB (SEQ ID NO:17)
HBKC	2E2 RHB (SEQ ID NO:3)	2E2 RKC (SEQ ID NO:18)
HBKD	2E2 RHB (SEQ ID NO:3)	2E2 RKD (SEQ ID NO:19)
HBKE	2E2 RHB (SEQ ID NO:3)	2E2 RKE (SEQ ID NO:20)
HBKF	2E2 RHB (SEQ ID NO:3)	2E2 RKF (SEQ ID NO:21)
HBKG	2E2 RHB (SEQ ID NO:3)	2E2 RKG (SEQ ID NO:22)
HCKA	2E2 RHC (SEQ ID NO:4)	2E2 RKA (SEQ ID NO:16)
HCKB	2E2 RHC (SEQ ID NO:4)	2E2 RKB (SEQ ID NO:17)
HCKC	2E2 RHC (SEQ ID NO:4)	2E2 RKC (SEQ ID NO:18)
HCKD	2E2 RHC (SEQ ID NO:4)	2E2 RKD (SEQ ID NO:19)
HCKE	2E2 RHC (SEQ ID NO:4)	2E2 RKE (SEQ ID NO:20)
HCKF	2E2 RHC (SEQ ID NO:4)	2E2 RKF (SEQ ID NO:21)
HCKG	2E2 RHC (SEQ ID NO:4)	2E2 RKG (SEQ ID NO:22)
HDKA	2E2 RHD (SEQ ID NO:5)	2E2 RKA (SEQ ID NO:16)
HDKB	2E2 RHD (SEQ ID NO:5)	2E2 RKB (SEQ ID NO:17)
HDKC	2E2 RHD (SEQ ID NO:5)	2E2 RKC (SEQ ID NO:18)
HDKD	2E2 RHD (SEQ ID NO:5)	2E2 RKD (SEQ ID NO:19)
HDKE	2E2 RHD (SEQ ID NO:5)	2E2 RKE (SEQ ID NO:20)

HDKF	2E2 RHD (SEQ ID NO:5)	2E2 RKF (SEQ ID NO:21)
HDKG	2E2 RHD (SEQ ID NO:5)	2E2 RKG (SEQ ID NO:22)
HEKA	2E2 RHE (SEQ ID NO:6)	2E2 RKA (SEQ ID NO:16)
HEKB	2E2 RHE (SEQ ID NO:6)	2E2 RKB (SEQ ID NO:17)
HEKC	2E2 RHE (SEQ ID NO:6)	2E2 RKC (SEQ ID NO:18)
HEKD	2E2 RHE (SEQ ID NO:6)	2E2 RKD (SEQ ID NO:19)
HEKE	2E2 RHE (SEQ ID NO:6)	2E2 RKE (SEQ ID NO:20)
HEKF	2E2 RHE (SEQ ID NO:6)	2E2 RKF (SEQ ID NO:21)
HEKG	2E2 RHE (SEQ ID NO:6)	2E2 RKG (SEQ ID NO:22)
HFKA	2E2 RHF (SEQ ID NO:7)	2E2 RKA (SEQ ID NO:16)
HFKB	2E2 RHF (SEQ ID NO:7)	2E2 RKB (SEQ ID NO:17)
HFKC	2E2 RHF (SEQ ID NO:7)	2E2 RKC (SEQ ID NO:18)
HFKD	2E2 RHF (SEQ ID NO:7)	2E2 RKD (SEQ ID NO:19)
HFKE	2E2 RHF (SEQ ID NO:7)	2E2 RKE (SEQ ID NO:20)
HFKF	2E2 RHF (SEQ ID NO:7)	2E2 RKF (SEQ ID NO:21)
HFKG	2E2 RHF (SEQ ID NO:7)	2E2 RKG (SEQ ID NO:22)
HGKA	2E2 RHG (SEQ ID NO:8)	2E2 RKA (SEQ ID NO:16)
HGKB	2E2 RHG (SEQ ID NO:8)	2E2 RKB (SEQ ID NO:17)
HGKC	2E2 RHG (SEQ ID NO:8)	2E2 RKC (SEQ ID NO:18)
HGKD	2E2 RHG (SEQ ID NO:8)	2E2 RKD (SEQ ID NO:19)
HGKE	2E2 RHG (SEQ ID NO:8)	2E2 RKE (SEQ ID NO:20)
HGKF	2E2 RHG (SEQ ID NO:8)	2E2 RKF (SEQ ID NO:21)
HGHG	2E2 RHG (SEQ ID NO:8)	2E2 RKG (SEQ ID NO:22)
HA2KA	2E2 RHA2 (SEQ ID NO:9)	2E2 RKA (SEQ ID NO:16)
HA2KB	2E2 RHA2 (SEQ ID NO:9)	2E2 RKB (SEQ ID NO:17)
HB2KA	2E2 RHB2 (SEQ ID NO:10)	2E2 RKA (SEQ ID NO:16)
HB2KB	2E2 RHB2 (SEQ ID NO:10)	2E2 RKB (SEQ ID NO:17)

HA2KF	2E2 RHA2 (SEQ ID NO:9)	2E2 RKF (SEQ ID NO:21)
HB2KF	2E2 RHB2 (SEQ ID NO:10)	2E2 RKF (SEQ ID NO:21)
HA2KC	2E2 RHA2 (SEQ ID NO:9)	2E2 RKC (SEQ ID NO:18)
HA2KD	2E2 RHA2 (SEQ ID NO:9)	2E2 RKD (SEQ ID NO:19)
HA2KE	2E2 RHA2 (SEQ ID NO:9)	2E2 RKE (SEQ ID NO:20)
HA2KF	2E2 RHA2 (SEQ ID NO:9)	2E2 RKF (SEQ ID NO:21)
HA2KG	2E2 RHA2 (SEQ ID NO:9)	2E2 RKG (SEQ ID NO:22)
HB2KC	2E2 RHB2 (SEQ ID NO:10)	2E2 RKC (SEQ ID NO:18)
HB2KD	2E2 RHB2 (SEQ ID NO:10)	2E2 RKD (SEQ ID NO:19)
HB2KE	2E2 RHB2 (SEQ ID NO:10)	2E2 RKE (SEQ ID NO:20)
HA2KFmut	2E2 RHA2 (SEQ ID NO:9)	2E2 RKF F-Y mut (SEQ ID NO:24)
HB2KFmut	2E2 RHB2 (SEQ ID NO:10)	2E2 RKF F-Y mut (SEQ ID NO:24)
HEKAmut	2E2 RHE (SEQ ID NO:6)	2E2 RKA F-Y mut (SEQ ID NO:23)
HEKFmut	2E2 RHE (SEQ ID NO:6)	2E2 RKF F-Y mut (SEQ ID NO:24)
HAKFmut	2E2 RHA (SEQ ID NO:2)	2E2 RKF F-Y mut (SEQ ID NO:24)
HBKFmut	2E2 RHB (SEQ ID NO:3)	2E2 RKF F-Y mut (SEQ ID NO:24)
HCKFmut	2E2 RHC (SEQ ID NO:4)	2E2 RKF F-Y mut (SEQ ID NO:24)
HDKFmut	2E2 RHD (SEQ ID NO:5)	2E2 RKF F-Y mut (SEQ ID NO:24)
HFKFmut	2E2 RHF (SEQ ID NO:7)	2E2 RKF F-Y mut (SEQ ID NO:24)
HGKFmut	2E2 RHG (SEQ ID NO:8)	2E2 RKF F-Y mut (SEQ ID NO:24)
RHE Y-VKA	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKA (SEQ ID NO:16)
RHE Y-VKB	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKB (SEQ ID NO:17)
RHE Y-VKC	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKC (SEQ ID NO:18)
RHE Y-VKD	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKD (SEQ ID NO:19)
RHE Y-VKE	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKE (SEQ ID NO:20)
RHE Y-VKF	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKF (SEQ ID NO:21)
RHE Y-VKG	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKG (SEQ ID NO:22)

RHE E-DKA	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKA (SEQ ID NO:16)
RHE E-DKB	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKB (SEQ ID NO:17)
RHE E-DKC	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKC (SEQ ID NO:18)
RHE E-DKD	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKD (SEQ ID NO:19)
RHE E-DKE	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKE (SEQ ID NO:20)
RHE E-DKF	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKF (SEQ ID NO:21)
RHE E-DKG	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKG (SEQ ID NO:22)
RHE E-DKFmut	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKF F-Y mut (SEQ ID NO:24)
RHE S-GKA	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKA (SEQ ID NO:16)
RHE S-GKB	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKB (SEQ ID NO:17)
RHE S-GKC	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKC (SEQ ID NO:18)
RHE S-GKD	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKD (SEQ ID NO:19)
RHE S-GKE	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKE (SEQ ID NO:20)
RHE S-GKF	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKF (SEQ ID NO:21)
RHE S-GKG	2E2 RHE S-G (SEQ ID NO:11)	2E2 RKG (SEQ ID NO:22)
RHE Triple-KA	2E2 RHE triple (SEQ ID NO:14)	2E2 RKA (SEQ ID NO:16)
RHE Triple-KB	2E2 RHE triple (SEQ ID NO:14)	2E2 RKB (SEQ ID NO:17)
RHE Triple-KC	2E2 RHE triple (SEQ ID NO:14)	2E2 RKC (SEQ ID NO:18)
RHE Triple-KD	2E2 RHE triple (SEQ ID NO:14)	2E2 RKD (SEQ ID NO:19)
RHE Triple-KE	2E2 RHE triple (SEQ ID NO:14)	2E2 RKE (SEQ ID NO:20)
RHE Triple-KF	2E2 RHE triple (SEQ ID NO:14)	2E2 RKF (SEQ ID NO:21)
RHE Triple-KG	2E2 RHE triple (SEQ ID NO:14)	2E2 RKG (SEQ ID NO:22)
RHE Triple-KFmut	2E2 RHE triple (SEQ ID NO:14)	2E2 RKF F-Y mut (SEQ ID NO:24)
RHE Y-VKFmut	2E2 RHE Y-V (SEQ ID NO:13)	2E2 RKF F-Y mut (SEQ ID NO:24)
RHE E-DKFmut	2E2 RHE E-D (SEQ ID NO:12)	2E2 RKF F-Y mut (SEQ ID NO:24)

[0156] There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α, δ, ε, γ and μ, respectively. The γ and α classes are further divided into

subclasses *e.g.*, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. IgG1 antibodies can exist in multiple polymorphic variants termed allotypes (reviewed in Jefferis and Lefranc 2009. mAbs Vol 1 Issue 4 1-7) any of which are suitable for use in some of the embodiments herein. Common allotypic variants in human populations are those designated by the letters a,f,n,z or combinations thereof. In any of the embodiments herein, the antibody may comprise a heavy chain Fc region comprising a human IgG Fc region. In further embodiments, the human IgG Fc region comprises a human IgG1 or IgG4. In some embodiments, the antibody is an IgG1 antibody. In some embodiments, the antibody is an IgG4 antibody. In some embodiments, the human IgG4 comprises the amino acid substitution S228P, wherein the amino acid residues are numbered according to the EU index as in Kabat. In some embodiments, the human IgG1 comprises the amino acid sequence of SEQ ID NO:78. In some embodiments, the human IgG4 comprises the amino acid sequence of SEQ ID NO:79.

[0157] In some embodiments, provided herein is an anti-Siglec-8 antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:75; and/or a light chain comprising the amino acid sequence selected from SEQ ID NOs:76 or 77. In some embodiments, the antibody may comprise a heavy chain comprising the amino acid sequence of SEQ ID NO:87; and/or a light chain comprising the amino acid sequence of SEQ ID NO:76. In some embodiments, the anti-Siglec-8 antibody is lirentelimab. In some embodiments, the anti-Siglec-8 antibody induces apoptosis of activated eosinophils. In some embodiments, the anti-Siglec-8 antibody induces apoptosis of resting eosinophils. In some embodiments, the anti-Siglec-8 antibody depletes activated eosinophils and inhibits mast cell activation. In some embodiments, the anti-Siglec-8 antibody depletes or reduces mast cells and inhibits mast cell activation. In some embodiments, the anti-Siglec-8 antibody depleted or reduces the number of mast cells. In some embodiments, the anti-Siglec-8 antibody kills mast cells by ADCC activity. In some embodiments, the antibody depletes or reduces mast cells expressing Siglec-8 in a tissue. In some embodiments, the antibody depletes or reduces mast cells expressing Siglec-8 in a biological fluid.

1. Antibody Affinity

[0158] In some aspects, an anti-Siglec-8 antibody described herein binds to human Siglec-8 with about the same or higher affinity and/or higher avidity as compared to mouse antibody 2E2 and/or mouse antibody 2C4. In certain embodiments, an anti-Siglec-8 antibody provided herein has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 150 nM, $\leq$ 100 nM, $\leq$ 50 nM, $\leq$ 10 nM, $\leq$ 1 nM,

≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10-8 M or less, e.g. from 10-8 M to 10-13 M, e.g., from 10-9 M to 10-13 M). In some embodiments, an anti-Siglec-8 antibody described herein binds to human Siglec-8 at about 1.5-fold, about 2- fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold or about 10-fold higher affinity than mouse antibody 2E2 and/or mouse antibody 2C4. In some embodiments, the anti-Siglec-8 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16 or 21.

[0159] In one embodiment, the binding affinity of the anti-Siglec-8 antibody can be determined by a surface plasmon resonance assay. For example, the Kd or Kd value can be measured by using a BIAcore™-2000 or a BIAcore™-3000 (BIAcore, Inc., Piscataway, N.J.) at 25° C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore® Inc.) are activated with N-ethyl-N′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Capture antibodies (e.g., anti-human-Fc) are diluted with 10 mM sodium acetate, pH 4.8, before injection at a flow rate of 30 µl/minute and further immobilized with an anti-Siglec-8 antibody. For kinetics measurements, two-fold serial dilutions of dimeric Siglec-8 are injected in PBS with 0.05% Tween 20 (PBST) at 25° C at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIAcore® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio koff/kon. See, e.g., Chen, Y., et al., (1999) J. Mol. Biol. 293:865-881.

[0160] In another embodiment, biolayer interferometry may be used to determine the affinity of anti-Siglec-8 antibodies against Siglec-8. In an exemplary assay, Siglec-8-Fc tagged protein is immobilized onto anti-human capture sensors, and incubated with increasing concentrations of mouse, chimeric, or humanized anti-Siglec-8 Fab fragments to obtain affinity measurements using an instrument such as, for example, the Octet Red 384 System (ForteBio).

[0161] The binding affinity of the anti-Siglec-8 antibody can, for example, also be determined by the Scatchard analysis described in Munson et al., Anal. Biochem., 107:220 (1980) using standard techniques well known in the relevant art. See also Scatchard, G., Ann. N.Y. Acad. Sci. 51:660 (1947).

2. Antibody Avidity

[0162] In some embodiments, the binding avidity of the anti-Siglec-8 antibody can be determined by a surface plasmon resonance assay. For example, the Kd or Kd value can be measured by using a BIAcore T100. Capture antibodies (e.g., goat-anti-human-Fc and goat-anti-mouse-Fc) are immobilized on a CM5 chip. Flow-cells can be immobilized with anti-human or with anti-mouse antibodies. The assay is conducted at a certain temperature and flow rate, for example, at 25oC at a flow rate of 30µl/min. Dimeric Siglec-8 is diluted in assay buffer at various concentrations, for example, at a concentration ranging from 15nM to 1.88pM. Antibodies are captured and high performance injections are conducted, followed by dissociations. Flow cells are regenerated with a buffer, for example, 50mM glycine pH 1.5. Results are blanked with an empty reference cell and multiple assay buffer injections, and analyzed with 1:1 global fit parameters.

3. Competition Assays

[0163] Competition-assays can be used to determine whether two antibodies bind the same epitope by recognizing identical or sterically overlapping epitopes or one antibody competitively inhibits binding of another antibody to the antigen. These assays are known in the art. Typically, antigen or antigen expressing cells is immobilized on a multi-well plate and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured. Common labels for such competition assays are radioactive labels or enzyme labels. In some embodiments, an anti-Siglec-8 antibody described herein competes with a 2E2 antibody described herein, for binding to the epitope present on the cell surface of a cell (*e.g.*, a mast cell). In some embodiments, an anti-Siglec-8 antibody described herein competes with an antibody comprising a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:1, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:15, for binding to the epitope present on the cell surface of a cell (*e.g.*, a mast cell). In some embodiments, an anti-Siglec-8 antibody described herein competes with a 2C4 antibody described herein, for binding to the epitope present on the cell surface of a cell (*e.g.*, a mast cell). In some embodiments, an anti-Siglec-8 antibody described herein competes with an antibody comprising a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:2 (as found in U.S. Pat. No. 8,207,305), and a light chain variable region comprising the amino acid sequence of SEQ ID NO:4 (as found in U.S. Pat. No. 8,207,305), for binding to the epitope present on the cell surface of a cell (*e.g.*, a mast cell).

4. Thermal Stability

[0164] In some aspects, an anti-Siglec-8 described herein has a melting temperature (Tm) of at least about 70°C, at least about 71°C, or at least about 72°C in a thermal shift assay. In an exemplary thermal shift assay, samples comprising a humanized anti-Siglec-8 antibody are incubated with a fluorescent dye (Sypro Orange) for 71 cycles with 1°C increase per cycle in a qPCR thermal cycler to determine the Tm. In some embodiments, the anti-Siglec-8 antibody has a similar or higher Tm as compared to mouse 2E2 antibody and/or mouse 2C4 antibody. In some embodiments, the anti-Siglec-8 antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16 or 21. In some embodiments, the anti-Siglec-8 antibody has the same or higher Tm as compared to a chimeric 2C4 antibody. In some embodiments, the anti-Siglec-8 antibody has the same or higher Tm as compared to an antibody having a heavy chain comprising the amino acid sequence of SEQ ID NO:84 and a light chain comprising the amino acid sequence of SEQ ID NO:85.

5. Biological Activity Assays

[0165] In some embodiments, an anti-Siglec-8 antibody described herein depletes eosinophils and inhibits mast cells. Assays for assessing apoptosis of cells are well known in the art, for example staining with Annexin V and the TUNNEL assay.

[0166] In some embodiments, an anti-Siglec-8 antibody described herein induces ADCC activity. In some embodiments, an anti-Siglec-8 antibody described herein kills eosinophils expressing Siglec-8 by ADCC activity. In some embodiments, a composition comprises non-fucosylated *(i.e.*, afucosylated) anti-Siglec-8 antibodies. In some embodiments, a composition comprising non-fucosylated anti-Siglec-8 antibodies described herein enhances ADCC activity against Siglec-8 expressing eosinophils as compared to a composition comprising partially fucosylated anti-Siglec-8 antibodies. Assays for assessing ADCC activity are well known in the art and described herein. In an exemplary assay, to measure ADCC activity, effector cells and target cells are used. Examples of effector cells include natural killer (NK) cells, large granular lymphocytes (LGL), lymphokine-activated killer (LAK) cells and PBMC comprising NK and LGL, or leukocytes having Fc receptors on the cell surfaces, such as neutrophils, eosinophils and macrophages. Effector cells can be isolated from any source including individuals with a disease of interest. The target cell is any cell which expresses on the cell surface antigens that antibodies to be evaluated can recognize. An example of such a target cell is an eosinophil which expresses

Siglec-8 on the cell surface. Another example of such a target cell is a cell line (*e.g.*, Ramos cell line) which expresses Siglec-8 on the cell surface (*e.g.*, Ramos 2C10)). Target cells can be labeled with a reagent that enables detection of cytolysis. Examples of reagents for labeling include a radio-active substance such as sodium chromate ($Na_2{}^{51}CrO_4$). See, *e.g.*, *Immunology*, 14, 181 (1968); *J. Immunol. Methods*., 172, 227 (1994); and *J. Immunol. Methods*., 184, 29 (1995).

[0167] In an exemplary assay to assess ADCC and apoptotic activity of anti-Siglec-8 antibodies on mast cells, human mast cells are isolated from human tissues or biological fluids according to published protocols (Guhl et al., *Biosci. Biotechnol. Biochem*., 2011, 75:382-384; Kulka et al., *In Current Protocols in Immunology*, 2001, (John Wiley & Sons, Inc.)) or differentiated from human hematopoietic stem cells, for example as described by Yokoi et al., *J Allergy Clin Immunol*., 2008, 121:499-505. Purified mast cells are resuspended in Complete RPMI medium in a sterile 96-well U-bottom plate and incubated in the presence or absence of anti-Siglec-8 antibodies for 30 minutes at concentrations ranging between 0.0001 ng/ml and 10 μg/ml. Samples are incubated for a further 4 to 48 hours with and without purified natural killer (NK) cells or fresh PBL to induce ADCC. Cell-killing by apoptosis or ADCC is analyzed by flow cytometry using fluorescent conjugated antibodies to detect mast cells (CD117 and FcεR1) and Annexin-V and 7AAD to discriminate live and dead or dying cells. Annexin-V and 7AAD staining are performed according to manufacturer's instructions.

[0168] In some aspects, an anti-Siglec-8 antibody described herein inhibits mast cell-mediated activities. Mast cell tryptase has been used as a biomarker for total mast cell number and activation. For example, total and active tryptase as well as histamine, N-methyl histamine, and 11-beta-prostaglandin F2 can be measured in blood or urine to assess the reduction in mast cells. See, *e.g.*, U.S. Patent Application Publication No. US 20110293631 for an exemplary mast cell activity assay.

E. Antibody Preparation

[0169] The antibody described herein (*e.g.*, an antibody that binds to human Siglec-8) is prepared using techniques available in the art for generating antibodies, exemplary methods of which are described in more detail in the following sections.

1. Antibody Fragments

[0170] The present disclosure encompasses antibody fragments. Antibody fragments may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. In

certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. For a review of certain antibody fragments, see Hudson et al. (2003) Nat. Med. 9:129-134.

[0171] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992); and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab′-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter et al., Bio/Technology 10: 163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Fab and $F(ab')_2$ fragment with increased in vivo half-life comprising salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv). *See* WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. Fv and scFv are the only species with intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during in vivo use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv. *See* Antibody Engineering, ed. Borrebaeck, supra. The antibody fragment may also be a "linear antibody", *e.g.*, as described in U.S. Pat. No. 5,641,870, for example. Such linear antibodies may be monospecific or bispecific.

2. Humanized Antibodies

[0172] The present disclosure encompasses humanized antibodies. Various methods for humanizing non-human antibodies are known in the art. For example, a humanized antibody can have one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter (Jones et al. (1986) Nature 321:522-525; Riechmann et al. (1988) Nature 332:323-327; Verhoeyen et al. (1988) Science 239:1534-1536), by substituting hypervariable region sequences for the corresponding sequences of a human antibody.

Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0173] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies can be important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent (*e.g.*, mouse) antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework for the humanized antibody (Sims et al. (1993) *J. Immunol.* 151:2296; Chothia et al. (1987) J. Mol. Biol. 196:901. Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al. (1992) Proc. Natl. Acad. Sci. USA, 89:4285; Presta et al. (1993) *J. Immunol.,* 151:2623.

[0174] It is further generally desirable that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to one method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those, skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

[0175] In certain embodiments, an antibody of the present disclosure is altered to increase or decrease the extent to which the antibody is glycosylated. Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of a carbohydrate moiety

to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0176] Addition or deletion of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that one or more of the above-described tripeptide sequences (for N-linked glycosylation sites) is created or removed. The alteration may also be made by the addition, deletion, or substitution of one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0177] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. For example, antibodies with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in US Pat Appl No US 2003/0157108 (Presta, L.). See also US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Antibodies with a bisecting N-acetylglucosamine (GlcNAc) in the carbohydrate attached to an Fc region of the antibody are referenced in WO 2003/011878, Jean-Mairet et al. and U.S. Pat. No. 6,602,684, Umana et al. Antibodies with at least one galactose residue in the oligosaccharide attached to an Fc region of the antibody are reported in WO 1997/30087, Patel et al. See, also, WO 1998/58964 (Raju, S.) and WO 1999/22764 (Raju, S.) concerning antibodies with altered carbohydrate attached to the Fc region thereof. See also US 2005/0123546 (Umana et al.) on antigen-binding molecules with modified glycosylation.

[0178] In certain embodiments, a glycosylation variant comprises an Fc region, wherein a carbohydrate structure attached to the Fc region lacks fucose. Such variants have improved ADCC function. Optionally, the Fc region further comprises one or more amino acid substitutions therein which further improve ADCC, for example, substitutions at positions 298, 333, and/or 334 of the Fc region (Eu numbering of residues). Examples of publications related to "defucosylated" or "fucose-deficient" antibodies include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; Okazaki et al. J. Mol. Biol. 336:1239-

1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004). Examples of cell lines producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004)), and cells overexpressing β1,4-N-acetylglycosminyltransferase III (GnT-III) and Golgi μ-mannosidase II (ManII).

[0179] Antibodies are contemplated herein that have reduced fucose relative to the amount of fucose on the same antibody produced in a wild-type CHO cell. For example, the antibody has a lower amount of fucose than it would otherwise have if produced by native CHO cells (e.g., a CHO cell that produce a native glycosylation pattern, such as, a CHO cell containing a native FUT8 gene). In certain embodiments, an anti-Siglec-8 antibody provided herein is one wherein less than about 50%, 40%, 30%, 20%, 10%, 5% or 1% of the N-linked glycans thereon comprise fucose. In certain embodiments, an anti-Siglec-8 antibody provided herein is one wherein none of the N-linked glycans thereon comprise fucose, i.e., wherein the antibody is completely without fucose, or has no fucose or is non-fucosylated or is afucosylated. The amount of fucose can be determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn297 (e.g., complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. In some embodiments, at least one or two of the heavy chains of the antibody is non-fucosylated.

[0180] In one embodiment, the antibody is altered to improve its serum half-life. To increase the serum half-life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Pat. No. 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG1, IgG2, IgG3, or IgG4) that is responsible for increasing the in vivo serum half-life of the IgG molecule (US 2003/0190311, U.S. Pat. No. 6,821,505; U.S. Pat. No. 6,165,745; U.S. Pat. No. 5,624,821; U.S. Pat. No. 5,648,260; U.S. Pat. No. 6,165,745; U.S. Pat. No. 5,834,597).

[0181] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by a different residue. Sites of interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 5 under the heading of "preferred substitutions." If such substitutions result in a desirable change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 5, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 5.

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0182] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or c) the bulk of the side chain. Amino acids may be grouped according to similarities in the properties of their

side chains (in A. L. Lehninger, in Biochemistry, second ed., pp. 73-75, Worth Publishers, New York (1975)):

(1) non-polar: Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

(2) uncharged polar: Gly (G), Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)

(3) acidic: Asp (D), Glu (E)

(4) basic: Lys (K), Arg (R), His (H)

[0183] Alternatively, naturally occurring residues may be divided into groups based on common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0184] Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, into the remaining (non-conserved) sites.

[0185] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have modified (e.g., improved) biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g., 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibodies thus generated are displayed from filamentous phage particles as fusions to at least part of a phage coat protein (e.g., the gene III product of M13) packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g., binding affinity). In order to identify candidate hypervariable region sites for modification, scanning mutagenesis (e.g., alanine scanning) can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to techniques known

in the art, including those elaborated herein. Once such variants are generated, the panel of variants is subjected to screening using techniques known in the art, including those described herein, and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0186] Nucleic acid molecules encoding amino acid sequence variants of the antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody.

[0187] It may be desirable to introduce one or more amino acid modifications in an Fc region of antibodies of the present disclosure, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g., a substitution) at one or more amino acid positions including that of a hinge cysteine. In some embodiments, the Fc region variant comprises a human IgG4 Fc region. In a further embodiment, the human IgG4 Fc region comprises the amino acid substitution S228P, wherein the amino acid residues are numbered according to the EU index as in Kabat.

[0188] In accordance with this description and the teachings of the art, it is contemplated that in some embodiments, an antibody of the present disclosure may comprise one or more alterations as compared to the wild type counterpart antibody, e.g. in the Fc region. These antibodies would nonetheless retain substantially the same characteristics required for therapeutic utility as compared to their wild type counterpart. For example, it is thought that certain alterations can be made in the Fc region that would result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in WO99/51642. See also Duncan & Winter Nature 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO94/29351 concerning other examples of Fc region variants. WO00/42072 (Presta) and WO 2004/056312 (Lowman) describe antibody variants with improved or diminished binding to FcRs. The content of these patent publications are specifically incorporated herein by reference. See, also, Shields et al. J. Biol. Chem. 9(2): 6591-6604 (2001). Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in

US2005/0014934A1 (Hinton et al.). These antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in U.S. Pat. No. 6,194,551B1, WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

7. Vectors, Host Cells, and Recombinant Methods

[0189] For recombinant production of an antibody of the present disclosure, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The choice of vector depends in part on the host cell to be used. Generally, host cells are of either prokaryotic or eukaryotic (generally mammalian) origin. It will be appreciated that constant regions of any isotype can be used for this purpose, including IgG, IgM, IgA, IgD, and IgE constant regions, and that such constant regions can be obtained from any human or animal species.

Generating Antibodies Using Prokaryotic Host Cells:

a) Vector Construction

[0190] Polynucleotide sequences encoding polypeptide components of the antibody of the present disclosure can be obtained using standard recombinant techniques. Desired polynucleotide sequences may be isolated and sequenced from antibody producing cells such as hybridoma cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in prokaryotic hosts. Many vectors that are available and known in the art can be used for the purpose of the present disclosure. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components, depending on its function (amplification or expression of heterologous polynucleotide, or both) and its compatibility with the particular host cell in which it resides. The vector components generally include, but are not limited to: an origin of replication, a selection marker gene, a promoter, a

ribosome binding site (RBS), a signal sequence, the heterologous nucleic acid insert and a transcription termination sequence.

[0191] In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, E. coli is typically transformed using pBR322, a plasmid derived from an E. coli species. pBR322 contains genes-encoding ampicillin (Amp) and tetracycline (Tet) resistance and thus provides easy means for identifying transformed cells. pBR322, its derivatives, or other microbial plasmids or bacteriophage may also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of endogenous proteins. Examples of pBR322 derivatives used for expression of particular antibodies are described in detail in Carter et al., U.S. Pat. No. 5,648,237.

[0192] In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, bacteriophage such as λGEM.TM.-11 may be utilized in making a recombinant vector which can be used to transform susceptible host cells such as E. coli LE392.

[0193] The expression vector of the present disclosure may comprise two or more promoter-cistron pairs, encoding each of the polypeptide components. A promoter is an untranslated regulatory sequence located upstream (5′) to a cistron that modulates its expression. Prokaryotic promoters typically fall into two classes, inducible and constitutive. Inducible promoter is a promoter that initiates increased levels of transcription of the cistron under its control in response to changes in the culture condition, e.g. the presence or absence of a nutrient or a change in temperature.

[0194] A large number of promoters recognized by a variety of potential host cells are well known. The selected promoter can be operably linked to cistron DNA encoding the light or heavy chain by removing the promoter from the source DNA via restriction enzyme digestion and inserting the isolated promoter sequence into the vector of the present disclosure. Both the native promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target genes. In some embodiments, heterologous promoters are utilized, as they generally permit greater transcription and higher yields of expressed target gene as compared to the native target polypeptide promoter.

[0195] Promoters suitable for use with prokaryotic hosts include the PhoA promoter, the β-galactamase and lactose promoter systems, a tryptophan (trp) promoter system and hybrid promoters such as the tac or the trc promoter. However, other promoters that are functional in bacteria (such as other known bacterial or phage promoters) are suitable as well. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to cistrons encoding the target light and heavy chains (Siebenlist et al. (1980) Cell 20: 269) using linkers or adaptors to supply any required restriction sites.

[0196] In one aspect of the present disclosure, each cistron within the recombinant vector comprises a secretion signal sequence component that directs translocation of the expressed polypeptides across a membrane. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. The signal sequence selected for the purpose of the present disclosure should be one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the signal sequences native to the heterologous polypeptides, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group consisting of the alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II (STII) leaders, LamB, PhoE, PelB, OmpA and MBP. In one embodiment of the present disclosure, the signal sequences used in both cistrons of the expression system are STII signal sequences or variants thereof.

[0197] In another aspect, the production of the immunoglobulins according to the present disclosure can occur in the cytoplasm of the host cell, and therefore does not require the presence of secretion signal sequences within each cistron. In that regard, immunoglobulin light and heavy chains are expressed, folded and assembled to form functional immunoglobulins within the cytoplasm. Certain host strains (e.g., the E. coli trxB-strains) provide cytoplasm conditions that are favorable for disulfide bond formation, thereby permitting proper folding and assembly of expressed protein subunits. Proba and Pluckthun Gene, 159:203 (1995).

[0198] Antibodies of the present disclosure can also be produced by using an expression system in which the quantitative ratio of expressed polypeptide components can be modulated in order to maximize the yield of secreted and properly assembled antibodies of the present disclosure. Such modulation is accomplished at least in part by simultaneously modulating translational strengths for the polypeptide components.

[0199] One technique for modulating translational strength is disclosed in Simmons et al., U.S. Pat. No. 5,840,523. It utilizes variants of the translational initiation region (TIR) within a cistron. For a given TIR, a series of amino acid or nucleic acid sequence variants can be created with a range of translational strengths, thereby providing a convenient means by which to adjust this factor for the desired expression level of the specific chain. TIR variants can be generated by conventional mutagenesis techniques that result in codon changes which can alter the amino acid sequence. In certain embodiments, changes in the nucleotide sequence are silent. Alterations in the TIR can include, for example, alterations in the number or spacing of Shine-Dalgarno sequences, along with alterations in the signal sequence. One method for generating mutant signal sequences is the generation of a "codon bank" at the beginning of a coding sequence that does not change the amino acid sequence of the signal sequence (i.e., the changes are silent). This can be accomplished by changing the third nucleotide position of each codon; additionally, some amino acids, such as leucine, serine, and arginine, have multiple first and second positions that can add complexity in making the bank. This method of mutagenesis is described in detail in Yansura et al. (1992) METHODS: A Companion to Methods in Enzymol. 4:151-158.

[0200] In one embodiment, a set of vectors is generated with a range of TIR strengths for each cistron therein. This limited set provides a comparison of expression levels of each chain as well as the yield of the desired antibody products under various TIR strength combinations. TIR strengths can be determined by quantifying the expression level of a reporter gene as described in detail in Simmons et al. U.S. Pat. No. 5,840,523. Based on the translational strength comparison, the desired individual TIRs are selected to be combined in the expression vector constructs of the present disclosure.

[0201] Prokaryotic host cells suitable for expressing antibodies of the present disclosure include Archaebacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include Escherichia (e.g., E. coli), Bacilli (e.g., B. subtilis), Enterobacteria, Pseudomonas species (e.g., P. aeruginosa), Salmonella typhimurium, Serratia marcescans, Klebsiella, Proteus, Shigella, Rhizobia, Vitreoscilla, or Paracoccus. In one embodiment, gram-negative cells are used. In one embodiment, E. coli cells are used as hosts for the present disclosure. Examples of E. coli strains include strain W3110 (Bachmann, Cellular and Molecular Biology, vol. 2 (Washington, D.C.: American Society for Microbiology, 1987), pp. 1190-1219; ATCC Deposit No. 27,325) and derivatives thereof, including strain 33D3 having genotype W3110 ΔfhuA (ΔtonA) ptr3 lac Iq lacL8 ΔompTΔ(nmpc-fepE) degP41 kanR

(U.S. Pat. No. 5,639,635). Other strains and derivatives thereof, such as E. coli 294 (ATCC 31,446), E. coli B, E. coliλ 1776 (ATCC 31,537) and E. coli RV308(ATCC 31,608) are also suitable. These examples are illustrative rather than limiting. Methods for constructing derivatives of any of the above-mentioned bacteria having defined genotypes are known in the art and described in, for example, Bass et al., Proteins, 8:309-314 (1990). It is generally necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, E. coli, Serratia, or Salmonella species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon. Typically the host cell should secrete minimal amounts of proteolytic enzymes, and additional protease inhibitors may desirably be incorporated in the cell culture.

b) Antibody Production

[0202] Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[0203] Transformation means introducing DNA into the prokaryotic host so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO. Yet another technique used is electroporation.

[0204] Prokaryotic cells used to produce the polypeptides of the present disclosure are grown in media known in the art and suitable for culture of the selected host cells. Examples of suitable media include luria broth (LB) plus necessary nutrient supplements. In some embodiments, the media also contains a selection agent, chosen based on the construction of the expression vector, to selectively permit growth of prokaryotic cells containing the expression vector. For example, ampicillin is added to media for growth of cells expressing ampicillin resistant gene.

[0205] Any necessary supplements besides carbon, nitrogen, and inorganic phosphate sources may also be included at appropriate concentrations introduced alone or as a mixture with another supplement or medium such as a complex nitrogen source. Optionally the culture medium may contain one or more reducing agents selected from the group consisting of glutathione, cysteine, cystamine, thioglycollate, dithioerythritol and dithiothreitol.

[0206] The prokaryotic host cells are cultured at suitable temperatures. In certain embodiments, for E. coli growth, growth temperatures range from about 20° C. to about 39° C.; from about 25° C. to about 37° C.; or about 30° C. The pH of the medium may be any pH ranging from about 5 to about 9, depending mainly on the host organism. In certain embodiments, for E. coli, the pH is from about 6.8 to about 7.4, or about 7.0.

[0207] If an inducible promoter is used in the expression vector of the present disclosure, protein expression is induced under conditions suitable for the activation of the promoter. In one aspect of the present disclosure, PhoA promoters are used for controlling transcription of the polypeptides. Accordingly, the transformed host cells are cultured in a phosphate-limiting medium for induction. In certain embodiments, the phosphate-limiting medium is the C.R.A.P. medium (see, e.g., Simmons et al., J. Immunol. Methods (2002), 263:133-147). A variety of other inducers may be used, according to the vector construct employed, as is known in the art.

[0208] In one embodiment, the expressed polypeptides of the present disclosure are secreted into and recovered from the periplasm of the host cells. Protein recovery typically involves disrupting the microorganism, generally by such means as osmotic shock, sonication or lysis. Once cells are disrupted, cell debris or whole cells may be removed by centrifugation or filtration. The proteins may be further purified, for example, by affinity resin chromatography. Alternatively, proteins can be transported into the culture media and isolated therein. Cells may be removed from the culture and the culture supernatant being filtered and concentrated for further purification of the proteins produced. The expressed polypeptides can be further isolated and identified using commonly known methods such as polyacrylamide gel electrophoresis (PAGE) and Western blot assay.

[0209] In one aspect of the present disclosure, antibody production is conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for production of recombinant proteins. Large-scale fermentations have at least 1000 liters of capacity, and in certain embodiments, about 1,000 to 100,000 liters of capacity. These fermentors use agitator impellers to distribute oxygen and nutrients, especially glucose. Small scale fermentation refers generally to fermentation in a fermentor that is no more than approximately 100 liters in volumetric capacity, and can range from about 1 liter to about 100 liters.

[0210] In a fermentation process, induction of protein expression is typically initiated after the cells have been grown under suitable conditions to a desired density, e.g., an OD550 of about

180-220, at which stage the cells are in the early stationary phase. A variety of inducers may be used, according to the vector construct employed, as is known in the art and described above. Cells may be grown for shorter periods prior to induction. Cells are usually induced for about 12-50 hours, although longer or shorter induction time may be used.

[0211] To improve the production yield and quality of the polypeptides of the present disclosure, various fermentation conditions can be modified. For example, to improve the proper assembly and folding of the secreted antibody polypeptides, additional vectors overexpressing chaperone proteins, such as Dsb proteins (DsbA, DsbB, DsbC, DsbD and or DsbG) or FkpA (a peptidylprolyl cis,trans-isomerase with chaperone activity) can be used to co-transform the host prokaryotic cells. The chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells. Chen et al. (1999) J. Biol. Chem. 274:19601-19605; Georgiou et al., U.S. Pat. No. 6,083,715; Georgiou et al., U.S. Pat. No. 6,027,888; Bothmann and Pluckthun (2000) J. Biol. Chem. 275:17100-17105; Ramm and Pluckthun (2000) J. Biol. Chem. 275:17106-17113; Arie et al. (2001) Mol. Microbiol. 39:199-210.

[0212] To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes can be used for the present disclosure. For example, host cell strains may be modified to effect genetic mutation(s) in the genes encoding known bacterial proteases such as Protease III, OmpT, DegP, Tsp, Protease I, Protease Mi, Protease V, Protease VI and combinations thereof. Some E. coli protease-deficient strains are available and described in, for example, Joly et al. (1998), supra; Georgiou et al., U.S. Pat. No. 5,264,365; Georgiou et al., U.S. Pat. No. 5,508,192; Hara et al., Microbial Drug Resistance, 2:63-72 (1996).

[0213] In one embodiment, E. coli strains deficient for proteolytic enzymes and transformed with plasmids overexpressing one or more chaperone proteins are used as host cells in the expression system of the present disclosure.

c) Antibody Purification

[0214] In one embodiment, the antibody protein produced herein is further purified to obtain preparations that are substantially homogeneous for further assays and uses. Standard protein purification methods known in the art can be employed. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on a cation-

exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, and gel filtration using, for example, Sephadex G-75.

[0215] In one aspect, Protein A immobilized on a solid phase is used for immunoaffinity purification of the antibody products of the present disclosure. Protein A is a 41 kD cell wall protein from Staphylococcus aureas which binds with a high affinity to the Fc region of antibodies. Lindmark et al (1983) J. Immunol. Meth. 62:1-13. The solid phase to which Protein A is immobilized can be a column comprising a glass or silica surface, or a controlled pore glass column or a silicic acid column. In some applications, the column is coated with a reagent, such as glycerol, to possibly prevent nonspecific adherence of contaminants.

[0216] As the first step of purification, a preparation derived from the cell culture as described above can be applied onto a Protein A immobilized solid phase to allow specific binding of the antibody of interest to Protein A. The solid phase would then be washed to remove contaminants non-specifically bound to the solid phase. Finally the antibody of interest is recovered from the solid phase by elution.

Generating Antibodies Using Eukaryotic Host Cells:

[0217] A vector for use in a eukaryotic host cell generally includes one or more of the following non-limiting components: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

a) Signal Sequence Component

[0218] A vector for use in a eukaryotic host cell may also contain a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide of interest. The heterologous signal sequence selected may be one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available. The DNA for such a precursor region is ligated in reading frame to DNA encoding the antibody.

b) Origin of Replication

[0219] Generally, an origin of replication component is not needed for mammalian expression vectors. For example, the SV40 origin may typically be used only because it contains the early promoter.

c) Selection Gene Component

[0220] Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, where relevant, or (c) supply critical nutrients not available from complex media.

[0221] One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

[0222] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the antibody nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -II, primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

[0223] For example, in some embodiments, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. In some embodiments, an appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (e.g., ATCC CRL-9096).

[0224] Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding an antibody, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3′-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199. Host cells may include NS0, CHOK1, CHOK1SV or derivatives, including cell lines deficient in glutamine synthetase (GS). Methods for the use of GS as a selectable marker for mammalian cells are described in U.S. Pat. No. 5,122,464 and U.S. Pat. No. 5,891,693.

d) Promoter Component

[0225] Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to nucleic acid encoding a polypeptide of interest (e.g., an antibody). Promoter sequences are known for eukaryotes. For example, virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3′

end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3′ end of the coding sequence. In certain embodiments, any or all of these sequences may be suitably inserted into eukaryotic expression vectors.

[0226] Transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[0227] The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Reyes et al., Nature 297:598-601 (1982), describing expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

e) Enhancer Element Component

[0228] Transcription of DNA encoding an antibody of the present disclosure by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the human cytomegalovirus early promoter enhancer, the mouse cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature 297:17-18 (1982) describing enhancer elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5′ or 3′ to the antibody polypeptide-encoding sequence, but is generally located at a site 5′ from the promoter.

f) Transcription Termination Component

[0229] Expression vectors used in eukaryotic host cells may also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly

available from the 5′ and, occasionally 3′, untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding an antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

g) Selection and Transformation of Host Cells

[0230] Suitable host cells for cloning or expressing the DNA in the vectors herein include higher eukaryote cells described herein, including vertebrate host cells. Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; CHOK1 cells, CHOK1SV cells or derivatives and a human hepatoma line (Hep G2).

[0231] Host cells are transformed with the above-described-expression or cloning vectors for antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

h) Culturing the Host Cells

[0232] The host cells used to produce an antibody of the present disclosure may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58:44 (1979), Barnes et al., Anal. Biochem. 102:255 (1980), U.S. Pat. No. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these

media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

i) Purification of Antibody

[0233] When using recombinant techniques, the antibody can be produced intracellularly, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, may be removed, for example, by centrifugation or ultrafiltration. Where the antibody is secreted into the medium, supernatants from such expression systems may be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis, and antibiotics may be included to prevent the growth of adventitious contaminants.

[0234] The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being a convenient technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., J. Immunol. Methods 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., EMBO J. 5:15671575 (1986)). The matrix to which the affinity ligand is attached may be agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABX™ resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™

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

[0235] Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to further purification, for example, by low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, performed at low salt concentrations (e.g., from about 0-0.25M salt).

[0236] In general, various methodologies for preparing antibodies for use in research, testing, and clinical use are well-established in the art, consistent with the above-described methodologies and/or as deemed appropriate by one skilled in the art for a particular antibody of interest.

Production of non-fucosylated antibodies

[0237] Provided herein are methods for preparing antibodies with a reduced degree of fucosylation. For example, methods contemplated herein include, but are not limited to, use of cell lines deficient in protein fucosylation (e.g., Lec13 CHO cells, alpha-1,6-fucosyltransferase gene knockout CHO cells, cells overexpressing β1,4-N-acetylglycosminyltransferase III and further overexpressing Golgi μ-mannosidase II, etc.), and addition of a fucose analog(s) in a cell culture medium used for the production of the antibodies. See Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; WO 2004/056312 A1; Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004); and US Pat. No. 8,574,907. Additional techniques for reducing the fucose content of antibodies include Glymaxx technology described in U.S. Patent Application Publication No. 2012/0214975. Additional techniques for reducing the fucose content of antibodies also include the addition of one or more glycosidase inhibitors in a cell culture medium used for the production of the antibodies. Glycosidase inhibitors include α-glucosidase I, α-glucosidase II, and α-mannosidase I. In some embodiments, the glycosidase inhibitor is an inhibitor of α-mannosidase I (e.g., kifunensine).

[0238] As used herein, "core fucosylation" refers to addition of fucose ("fucosylation") to N-acetylglucosamine ("GlcNAc") at the reducing terminal of an N-linked glycan. Also provided are antibodies produced by such methods and compositions thereof.

[0239] In some embodiments, fucosylation of complex N-glycoside-linked sugar chains bound to the Fc region (or domain) is reduced. As used herein, a "complex N-glycoside-linked sugar chain" is typically bound to asparagine 297 (according to the number of Kabat), although a

complex N-glycoside linked sugar chain can also be linked to other asparagine residues. A "complex N-glycoside-linked sugar chain" excludes a high mannose type of sugar chain, in which only mannose is incorporated at the non-reducing terminal of the core structure, but includes 1) a complex type, in which the non-reducing terminal side of the core structure has one or more branches of galactose-N-acetylglucosamine (also referred to as "gal-GlcNAc") and the non-reducing terminal side of Gal-GlcNAc optionally has a sialic acid, bisecting N-acetylglucosamine or the like; or 2) a hybrid type, in which the non-reducing terminal side of the core structure has both branches of the high mannose N-glycoside-linked sugar chain and complex N-glycoside-linked sugar chain.

[0240] In some embodiments, the "complex N-glycoside-linked sugar chain" includes a complex type in which the non-reducing terminal side of the core structure has zero, one or more branches of galactose-N-acetylglucosamine (also referred to as "gal-GlcNAc") and the non-reducing terminal side of Gal-GlcNAc optionally further has a structure such as a sialic acid, bisecting N-acetylglucosamine or the like.

[0241] According to the present methods, typically only a minor amount of fucose is incorporated into the complex N-glycoside-linked sugar chain(s). For example, in various embodiments, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 1% of the antibody has core fucosylation by fucose in a composition. In some embodiments, substantially none (i.e., less than about 0.5%) of the antibody has core fucosylation by fucose in a composition. In some embodiments, more than about 40%, more than about 50%, more than about 60%, more than about 70%, more than about 80%, more than about 90%, more than about 91%, more than about 92%, more than about 93%, more than about 94%, more than about 95%, more than about 96%, more than about 97%, more than about 98%, or more than about 99% of the antibody is nonfucosylated in a composition.

[0242] In some embodiments, provided herein is an antibody wherein substantially none (i.e., less than about 0.5%) of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some embodiments, provided herein is an antibody wherein at least one or two of the heavy chains of the antibody is non-fucosylated.

[0243] As described above, a variety of mammalian host-expression vector systems can be utilized to express an antibody. In some embodiments, the culture media is not supplemented with fucose. In some embodiments, an effective amount of a fucose analog is added to the

culture media. In this context, an "effective amount" refers to an amount of the analog that is sufficient to decrease fucose incorporation into a complex N-glycoside-linked sugar chain of an antibody by at least about 10%, at least about 20%, at least about 30%, at least about 40% or at least about 50%. In some embodiments, antibodies produced by the instant methods comprise at least about 10%, at least about 20%, at least about 30%, at least about 40% or at least about 50% non-core fucosylated protein (e.g., lacking core fucosylation), as compared with antibodies produced from the host cells cultured in the absence of a fucose analog.

[0244] The content (e.g., the ratio) of sugar chains in which fucose is not bound to N-acetylglucosamine in the reducing end of the sugar chain versus sugar chains in which fucose is bound to N-acetylglucosamine in the reducing end of the sugar chain can be determined, for example, as described in the Examples. Other methods include hydrazinolysis or enzyme digestion (see, e.g., Biochemical Experimentation Methods 23: Method for Studying Glycoprotein Sugar Chain (Japan Scientific Societies Press), edited by Reiko Takahashi (1989)), fluorescence labeling or radioisotope labeling of the released sugar chain and then separating the labeled sugar chain by chromatography. Also, the compositions of the released sugar chains can be determined by analyzing the chains by the HPAEC-PAD method (see, e.g., J. Liq Chromatogr. 6:1557 (1983)). (See generally U.S. Patent Application Publication No. 2004/0110282.).

III. Articles of Manufacture or Kits

[0245] In another aspect, an article of manufacture or kit is provided which comprises a liquid formulation or pharmaceutical composition of the present disclosure comprising an anti-Siglec-8 antibody described herein (e.g., an antibody that binds human Siglec-8). The article of manufacture or kit may further comprise instructions for use of the antibody in the methods of the present disclosure, *e.g.*, for subcutaneous administration. In certain embodiments, the individual is a human.

[0246] The article of manufacture or kit may further comprise a container. Suitable containers include, for example, bottles, vials (e.g., dual chamber vials), syringes (such as single or dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the formulation. In some embodiments, the container is a glass vial.

[0247] The article of manufacture or kit may further comprise a label or a package insert, which is on or associated with the container, may indicate directions for reconstitution and/or use of the formulation. The label or package insert may further indicate that the formulation is useful or intended for subcutaneous administration for treating and/or preventing a disease or disorder of the present disclosure in an individual. For example, in some embodiments, the label or package insert may further indicate that the formulation is useful or intended for subcutaneous administration for treating and/or preventing one or more diseases or disorders selected from the group consisting of: chronic rhinosinusitis with concomitant asthma, aspirin-exacerbated respiratory disease, adult onset non-atopic asthma with sinus disease, chronic obstructive pulmonary disease, fibrotic disease, pre-fibrotic disease, advanced systemic mastocytosis, indolent systemic mastocytosis (ISM), inflammatory bowel disease (IBD), eosinophilic esophagitis (EOE), eosinophilic gastritis (EG), eosinophilic gastroenteritis (EGE), eosinophilic colitis (EOC), eosinophilic duodenitis, mast cell gastritis or mast cell gastroenteritis, gastritis or gastroenteritis with elevated mast cells, irritable bowel syndrome, irritable bowel syndrome with elevated mast cells, functional gastrointestinal disease, functional dyspepsia, allergic conjunctivitis, giant papillary conjunctivitis, chronic urticaria, allergic bronchopulmonary aspergillosis (ABPA), allergic asthma, asthma with eosinophil or mast cell phenotype, eosinophilic granulomatosis with polyangiitis (EGPA), celiac disease, gastroparesis, hypereosinophilic syndrome, atopic dermatitis, anaphylaxis, angioedema, mast cell activation syndrome/disorder, and eosinophilic fasciitis.

[0248] The container holding the formulation may be a single-use vial or a multi-use vial, which allows for repeat administrations of the reconstituted formulation. The article of manufacture or kit may further comprise a second container comprising a suitable diluent. The article of manufacture or kit may further include other materials desirable from a commercial, therapeutic, and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[0249] In a specific embodiment, the present disclosure provides kits for a single dose-administration unit. Such kits comprise a container of an aqueous formulation of therapeutic antibody, including both single or multi-chambered pre-filled syringes. Exemplary pre-filled syringes are available from Vetter GmbH, Ravensburg, Germany.

[0250] In another embodiment, provided herein is an article of manufacture or kit comprising the formulations described herein for administration in an auto-injector device. An auto-injector

can be described as an injection device that upon activation, will deliver its contents without additional necessary action from the patient or administrator. They are particularly suited for self-medication of therapeutic formulations when the delivery rate must be constant and the time of delivery is greater than a few moments.

[0251] It is understood that the aspects and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

EXAMPLES

[0252] The present disclosure will be more fully understood by reference to the following examples. The examples should not, however, be construed as limiting the scope of the present disclosure. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: pH screening for subcutaneous anti-Siglec-8 antibody formulations

[0253] The following Examples describe experiments aimed at identifying the appropriate formulation conditions for subcutaneous administration of the anti-Siglec-8 antibody AK002 (a humanized, afucosylated antibody comprising a heavy chain comprising the sequence of SEQ ID NO:75 and a light chain comprising the sequence of SEQ ID NO:76). Current commercial products at high concentrations are typically formulated in histidine buffers with pH conditions of 5.5-6.3 and sugar concentrations of 5-9%. As a preliminary high concentration formulation study for the anti-Siglec-8 antibody, various buffer solutions were used based on their pKa to screen the desired pH range. Excipient addition was also evaluated comparing effects of sugars, salt and arginine. The success of the formulation was based on maintaining the high concentration product in solution for a given period of time with little to no increase in product turbidity or aggregate formation.

Materials and Methods

[0254] The starting material used in the subcutaneous formulation development studies was a frozen GMP drug substance (DS). The DS was thawed at ambient temperature before reprocessing. The thawed DS was re-purified to remove the Polysorbate 80 prior to beginning the studies.

[0255] Based on the study performed, aliquots of the reprocessed material were dialyzed into the appropriate buffer being tested using 10K molecular weight cut off (MWCO) dialysis cassettes. The dialyzed material was transferred to Amicon centrifugation filters with 30K MWCO. Product pools were concentrated to the point where turbidity was visible or in the absence of turbidity, the desired concentration was achieved using a table top Eppendorf centrifuge operated at 3000 rcf. Final product concentrations were analyzed by UV absorbance at 280nm using a Perkin Elmer spectrophotometer. Sodium chloride, arginine, sucrose and trehalose excipients were added to evaluate excipient effects on concentrated samples. Turbidity was analyzed by UV absorbance at 340nm also using a Perkin Elmer spectrophotometer. The pH of the concentrated pools were confirmed using a Mettler Toledo pH meter and the samples were visually evaluated using polystyrene cuvettes. Based on the success of the formulation, some pools were analyzed by analytical Size Exclusion Chromatography (SEC) to evaluate monomer content. Concentrated pools were stored in a refrigerator at 2-8 ºC to evaluate temperature effect on samples. Sample pools that showed greatest product stability following storage at 2-8 ºC would be further evaluated in subsequent studies.

[0256] Concentration of the antibody material at varying pH allowed visual evaluation of turbidity. Turbidity was further confirmed by light scattering analysis at UV absorbance of 340nm. Samples were also analyzed by Size Exclusion Chromatography (SEC) to evaluate monomer and aggregate content.

Results

[0257] A pH screening range of 5.0 to 7.2 was tested using various buffers. 15mM potassium phosphate buffer was used to evaluate the anti-Siglec-8 product at pH 7.2. The potassium phosphate pool was concentrated from 11.0 mg/ml to 110 mg/ml (10x concentration factor). During concentration, turbidity was observed at approximately 32 mg/ml and became progressively more turbid as pool concentration increased. **FIG. 1** shows turbidity transition during potassium phosphate pool concentration.

[0258] 15mM L-histidine buffer was used to evaluate the anti-Siglec-8 product at pH 6.4. The L-histidine pool was concentrated from 11.0 mg/ml to 185 mg/ml (17x concentration factor). During concentration, no turbidity was observed but product showed indications of high viscosity at 185 mg/ml. Following 1 hour of exposure at ambient temperature, solidification or gelling of the 185 mg/ml sample was occurring. After overnight storage at ambient temperature, the 15mM L-histidine pool at 185 mg/ml had completely gelled. **FIG. 2** shows turbidity transition during histidine pool concentration.

[0259] 15mM sodium succinate buffer was used to evaluate the anti-Siglec-8 product at pH 6.0. The sodium succinate pool was concentrated from 18.0 mg/ml to 170 mg/ml (9x concentration factor). During concentration, turbidity was observed at approximately 120 mg/ml and became progressively more turbid as pool concentration increased. Following 1 hour at ambient temperature, formation of gel occurred. **FIG. 3** shows turbidity transition during sodium succinate pH 6.0 pool concentration.

[0260] 15mM sodium succinate buffer was used to evaluate the anti-Siglec-8 product at pH 5.6 also. The sodium succinate pool was concentrated from 10.0 mg/ml to 165 mg/ml (16x concentration factor). During concentration, turbidity was observed at approximately 125 mg/ml and became progressively more turbid as pool concentration increased. Heavy turbidity was apparent after 1 hour at ambient temperature and formation of gel occurred after 3 days at ambient temperature. **FIG. 4** shows turbidity transition during sodium succinate pH 5.6 pool concentration.

[0261] 15mM sodium acetate buffer was used to evaluate the anti-Siglec-8 product at pH 5.0. The sodium acetate pool was concentrated from 17.0 mg/ml to 190 mg/ml (11x concentration factor). During concentration, no turbidity was observed but product showed indications of moderate viscosity at 190 mg/ml. See **FIG. 5** for product transition during sodium acetate concentration. No presence of turbidity or gelling was observed in the 190 mg/ml sample at up to 7 days at 5°C. See Table A for all sample conditions and results.

Table A. pH screening results.

Buffer	pH	Starting Concentration (mg/mL)	Final Concentration (mg/mL)	Visual Observation at Final Concentration
15mM Potassium Phosphate	7.2	11.0	110.0	High turbidity

15mM L-Histidine	6.4	11.0	185.0	Clear, light yellow in color
15mM Sodium Succinate	6.0	18.0	170.0	High turbidity
15mM Sodium Succinate	5.6	10.0	165.0	High turbidity
15mM Sodium Acetate	5.0	17.0	190.0	Clear, light yellow in color

[0262] In conclusion, sodium acetate, sodium succinate, histidine, and potassium phosphate buffers were used to evaluate pH range based on their pKa. Histidine and sodium acetate buffers allowed for anti-Siglec-8 antibody concentration without turbidity. Antibody was found to form gels at high concentration in sodium succinate buffer at pH 5.6 or 6.0.

Example 2: Excipient screening for subcutaneous anti-Siglec-8 antibody formulations

[0263] In this Example, excipients were added to various buffer solutions to evaluate turbidity as described in Example 1 above.

[0264] Anti-Siglec-8 antibody in 15mM potassium phosphate buffer pH 7.2 was concentrated to 110 mg/ml. Arginine was added to the first sample to achieve a concentration of 320mM. At a final concentration of 85mg/ml, the pool was clear at time zero and at day 3 at both ambient temperature and 5 °C. Sucrose was added to the second sample to achieve a concentration of 540mM. At a final concentration of 70mg/ml, the pool was moderately turbid at time zero. Sodium chloride was added to the third sample to achieve a concentration of 500mM. At a final concentration of 80mg/ml, the pool was heavily turbid at time zero. **FIG. 6** shows comparison of turbidity between the four samples at pH 7.2.

[0265] Anti-Siglec-8 antibody in 15mM histidine buffer pH 6.4 was concentrated to 185 mg/ml. Arginine was added to the first sample to achieve a concentration of 100mM. At a final concentration of 165 mg/ml, the pool was moderately turbid at time zero and heavily turbid at day 1 with onset of gelling. Sucrose was added to the second sample to achieve a concentration of 260mM. At a final concentration of 150 mg/ml, the pool was moderately turbid at time zero and clear after 1 day of incubation at ambient temperature. The sucrose pool remained clear for >1 month at 5 °C. Sodium chloride was added to the third sample to achieve a concentration of 140mM. At a final concentration of 165mg/ml, the pool was heavily turbid at time zero and day 1 at ambient temperature with onset of gelling. **FIG. 7** shows comparison of turbidity between the four samples at pH 6.4.

[0266] Anti-Siglec-8 antibody in 15mM sodium succinate buffer pH 5.6 was concentrated to 165 mg/ml. Arginine was added to the first sample to achieve a concentration of 100mM. At a final concentration of 150 mg/ml, the pool was slightly turbid at time zero and moderately turbid at day 3 at ambient temperature. Sucrose was added to the second sample to achieve a concentration of 260mM. At a final concentration of 130 mg/ml, the pool was clear at time zero and clear after day 3 at ambient temperature. Sodium chloride was added to the third sample to achieve a concentration of 140mM. At a final concentration of 150 mg/ml, the pool was heavily turbid at time zero with onset of gelling at day 3 at ambient temperature. **FIG. 8** shows comparison of turbidity between the four samples at pH 5.6.

[0267] Anti-Siglec-8 antibody in 15mM sodium acetate buffer pH 5.0 was concentrated to 190 mg/ml. Arginine was added to the first sample to achieve a concentration of 100mM. At a final concentration of 170 mg/ml, the pool was clear at time zero and remained clear through day 7 at 5 ºC. Sucrose was added to the second sample to achieve a concentration of 260mM. At a final concentration of 155 mg/ml, the pool was clear at time zero and remained clear through day 7 at 5 ºC. Sodium chloride was added to the third sample to achieve a concentration of 140mM. At a final concentration of 170 mg/ml, the pool was clear at time zero and remained clear through day 7 at 5 ºC. Table B includes the results from the excipient addition studies.

Table B. Excipient addition results

Buffer	Excipient	Final Concentration (mg/mL)	Visual Observation at Final Concentration
15mM Potassium Phosphate, pH 7.2	Arginine, 320mM	85.0	Clear up to 3 days at 5 °C
	Sucrose, 540mM	70.0	Moderate turbidity at T=0
	Sodium Chloride, 500mM	80.0	Heavy turbidity at T=0
15mM L-Histidine, pH 6.4	Arginine, 100mM	165.0	Moderate turbidity at T=0
	Sucrose, 260mM	150.0	Clear for 1 month at 5 °C
	Sodium Chloride, 140mM	165.0	Heavy turbidity at T=0
15mM Sodium Succinate, pH 5.6	Arginine, 100mM	150.0	Slight turbidity at T=0
	Sucrose, 260mM	130.0	Clear up to 3 days at RT
	Sodium Chloride, 140mM	150.0	Heavy turbidity at T=0
15mM Sodium Acetate, pH 5.0	Arginine, 100mM	170.0	Clear up to 7 days at 5 °C
	Sucrose, 260mM	155.0	Clear up to 7 days at 5 °C

	Sodium Chloride, 140mM	170.0	Clear up to 7 days at 5 °C

Example 3: Screening combinations of pH and excipients for subcutaneous anti-Siglec-8 antibody formulations

[0268] Based on the positive buffer and excipient data from the previous studies, excipient addition of sucrose and trehalose was combined with pH ranging using sodium acetate and histidine to evaluate turbidity. 5mM sodium acetate with 260mM sucrose or 260mM trehalose was evaluated at pH 5.0, 5.3 and 5.6. 15mM histidine with 260mM sucrose or 260mM trehalose was evaluated at pH 5.5, 5.8 and 6.1.

[0269] Three aliquots of anti-Siglec-8 antibody in 15mM sodium acetate at pH 5.0, 5.3 and 5.6 were concentrated from 11.0 mg/ml to 175 mg/ml. Following pH analysis, it was noted that the pH of each pool had increased ~0.2-0.3 units suggesting that the Gibbs-Donnan effect had occurred. The control samples (pH 5.3, pH 5.6 and pH 5.8 at ~175 mg/ml) were set aside at ambient temperature. Six study samples were generated in total. Two samples at pH 5.3, two at pH 5.6 and two at pH 5.8. Sucrose was added to the first set of samples (pH 5.3, 5.6 and 5.8) to achieve a concentration of 260mM. Final concentrations for all sucrose study samples were ~140 mg/ml. Samples were stored at 5 °C to evaluate turbidity over time. All sucrose samples remained clear through day 14 at 5 °C. Trehalose was added to the second set of samples (pH 5.3, 5.6 and 5.8) to achieve a concentration of 260mM. Final concentrations for all trehalose study samples were ~130 mg/ml. Samples were stored at 5 °C to evaluate turbidity over time. All trehalose samples remained clear through day 14 at 5 °C. Visual observations of all sodium acetate samples generated are shown in Table C.

Table C. Sodium acetate excipient and pH ranging visual results

Buffer	Excipient	Final Concentration (mg/mL)	Visual Observation at Final Concentration
15mM Sodium Acetate, pH 5.3	260mM Sucrose	140.0	Clear up to 14 days at 5 °C
15mM Sodium Acetate, pH 5.6	260mM Sucrose	140.0	Clear up to 14 days at 5 °C
15mM Sodium Acetate, pH 5.8	260mM Sucrose	140.0	Clear up to 14 days at 5 °C
15mM Sodium Acetate,	260mM	130.0	Clear up to 14 days at

pH 5.3	Trehalose		5 °C
15mM Sodium Acetate, pH 5.6	260mM Trehalose	130.0	Clear up to 14 days at 5 °C
15mM Sodium Acetate, pH 5.8	260mM Trehalose	130.0	Clear up to 14 days at 5 °C

[0270] Sodium acetate sample turbidity was analyzed by a light scattering method using UV absorbance at 340nm. Results of the sodium acetate light scattering analysis are shown in Table D.

Table D. Sodium acetate excipient and pH ranging light scattering results

Sample Description	T=0	Day 3, RT	Day 14, 2-8 C
Acetate Control, pH 5.3	0.397	0.382	0.406
Acetate/Sucrose, pH 5.3	0.329	0.324	0.374
Acetate/Trehalose, pH 5.3	0.314	0.313	0.334
Acetate Control, pH 5.6	0.426	0.425	0.472
Acetate/Sucrose, pH 5.6	0.362	0.370	0.400
Acetate/Trehalose, pH 5.6	0.335	0.333	0.358
Acetate Control, pH 5.8	0.409	0.429	0.442
Acetate/Sucrose, pH 5.8	0.344	0.337	0.364
Acetate/Trehalose, pH 5.8	0.319	0.326	0.351

[0271] Three aliquots of anti-Siglec-8 antibody in 15mM histidine at pH 5.5, 5.8 and 6.1 were concentrated from 11.0 mg/ml to 175 mg/ml. Following pH analysis, there was little indication of pH change after concentration. The control samples (pH 5.5, pH 5.8 and pH 6.1 at ~175 mg/ml) were set aside at ambient temperature. Six study samples were generated in total. Two samples at pH 5.5, two at pH 5.8 and two at pH 6.1. Sucrose was added to the first set of samples (pH 5.5, 5.8 and 6.1) to achieve a concentration of 260mM. Final concentrations for all sucrose study samples were ~142 mg/ml. Samples were stored at 5 °C to evaluate turbidity over time. All sucrose samples remained clear through day 14 at 5 °C. Trehalose was added to the second set of samples (pH 5.5, 5.8 and 6.1) to achieve a concentration of 260mM. Final concentrations for all trehalose study samples were ~132 mg/ml. Samples were stored at 5 °C to evaluate turbidity over time. All trehalose samples remained clear through day 14 at 5 °C. Visual observations of all histidine samples generated are shown in Table E.

Table E. Histidine excipient and pH ranging visual results

Buffer	Excipient	Final Concentration (mg/mL)	Visual Observation at Final Concentration
15mM Histidine, pH 5.5	260mM Sucrose	142.0	Clear up to 14 days at 5 °C
15mM Histidine, pH 5.8	260mM Sucrose	142.0	Clear up to 14 days at 5 °C
15mM Histidine, pH 6.1	260mM Sucrose	142.0	Clear up to 14 days at 5 °C
15mM Histidine, pH 5.5	260mM Trehalose	132.0	Clear up to 14 days at 5 °C
15mM Histidine, pH 5.8	260mM Trehalose	132.0	Clear up to 14 days at 5 °C
15mM Histidine, pH 6.1	260mM Trehalose	132.0	Clear up to 14 days at 5 °C

[0272] Histidine sample turbidity was analyzed by a light scattering method using UV absorbance at 340nm. Results of the histidine light scattering analysis are shown in Table F.

Table F. Histidine excipient and pH ranging light scattering results

Sample Description	T=0	Day 2, RT	Day 14, 2-8 C
Histidine Control, pH 5.5	0.411	0.415	0.448
Histidine/Sucrose, pH 5.5	0.356	0.344	0.378
Histidine/Trehalose, pH 5.5	0.329	0.325	0.359
Histidine Control, pH 5.8	0.424	0.436	0.511
Histidine/Sucrose, pH 5.8	0.351	0.359	0.416
Histidine/Trehalose, pH 5.8	0.350	0.340	0.390
Histidine Control, pH 6.1	0.419	0.437	0.495
Histidine/Sucrose, pH 6.1	0.368	0.380	0.408
Histidine/Trehalose, pH 6.1	0.344	0.340	0.399

[0273] Histidine samples were analyzed for monomer content using analytical Size Exclusion Chromatography (SEC). Results of the histidine SEC analysis are shown in Table G.

Table G. Histidine excipient and pH ranging SEC results

Sample Description	Time Point	Monomer (%)	Aggregate (%)
Histidine Control pH 5.5	T=0	99.43	0.58

Histidine + 260mM Sucrose pH 5.5	Day 14 5C	99.39	0.61
Histidine + 260mM Trehalose pH 5.5	Day 14 5C	99.39	0.61
Histidine Control pH 5.8	T=0	99.42	0.59
Histidine + 260mM Sucrose pH 5.8	Day 14 5C	99.38	0.62
Histidine + 260mM Trehalose pH 5.8	Day 14 5C	99.37	0.63
Histidine Control pH 6.1	T=0	99.42	0.58
Histidine + 260mM Sucrose pH 6.1	Day 14 5C	99.35	0.65
Histidine + 260mM Trehalose pH 6.1	Day 14 5C	99.37	0.63

[0274] Studies were performed to confirm the Gibbs-Donnan effect observed during previous experiments. In the first study, anti-Siglec-8 antibody in 15mM sodium acetate pH 5.20 was concentrated to ~210 mg/ml. Following concentration, the pool resulted in a pH of 5.44, an increase of 0.24 pH units. In the second study, anti-Siglec-8 antibody in 15mM histidine pH 5.70 was concentrated to ~195 mg/ml. Following concentration, the pool resulted in a pH of 5.89, an increase of 0.19 pH units. The pH results are shown in Table H.

Table H. Histidine excipient and pH ranging results

Buffer	Initial concentration (mg/ml)	Final concentration (mg/ml)	Initial pH	Final pH	pH variation
15mM Sodium Acetate	18.0	210.0	5.20	5.44	0.24
15mM Histidine	18.0	195.0	5.70	5.89	0.19

Conclusions

[0275] The pH screening studies suggested that the anti-Siglec-8 antibody molecule is more stable in the pH range of 5.0-6.4. Evaluation of the pH range indicated that the antibody falls out of solution at pH≥6.4, regardless of buffer. In buffer pH 7.2, the molecule began precipitating as turbidity was observed at a concentration of 32 mg/mL and progressively worsened with further concentration. The antibody molecule also has a preference for specific buffer solutions. As indicated in Table A, the molecule at high concentration (>165mg/mL) remained in solution for

an extended period of time in 15mM acetate pH 5.0 and 15mM L-histidine pH 6.4 but precipitated in 15mM succinate buffer at pH 5.6 and 6.0.

[0276] With the addition of specific excipients, the stability of the molecule at high concentration improved. At pH 5.0, 5.6 and 6.4, high concentration pools (≥130 mg/mL) in the presence of 260mM sucrose remained clear for an extended period of time. Further experimentation confirmed that the anti-Siglec-8 antibody molecule at high concentration (≥130 mg/mL) in the presence of sugars (260mM sucrose and 260 mM trehalose) remained clear at 5 °C for > 14 days. Light scattering results showed decreased absorbance at 340 nanometers for samples containing sugars compared to control sample without sugars. SEC analytical results also confirmed the stability of the molecule as monomer content was unchanged after 14 days of storage at 5 °C. The Gibbs-Donnan effect was confirmed during the high concentration studies and became more evident as higher product concentrations were achieved. Surprisingly, a salting out effect was observed in the presence of arginine at high concentration, and also observed in the presence of sodium chloride. The most successful conditions were a pH of 5.0-6.3, histidine or sodium acetate buffer, sucrose at 5% to 9%, trehalose at 4% to 10%, and an antibody concentration ≥150mg/mL.

[0277] The information provided by these initial high concentration formulation development studies suggests formulating the anti-Siglec-8 antibody molecule within the pH range of 5.0 to 6.4 using sodium acetate or L-histidine buffers in the presence of sugars to attain desired long-term stability.

Example 4: A Phase I study to evaluate safety, tolerability, and bioavailability of subcutaneously administered anti-Siglec-8 antibody in adult healthy volunteers

[0278] The anti-Siglec-8 antibody described in Examples 1 and 2, administered as an intravenous infusion every 4 weeks, has previously been tested in healthy volunteers and in subjects with indolent systemic mastocytosis (ISM), chronic urticaria, severe allergic conjunctivitis (AC), and eosinophilic gastritis (EG) and/or eosinophilic duodenitis (EoD), previously referred to as eosinophilic gastroenteritis (EGE). Multiple doses of 3 mg/kg have been given to subjects with ISM, urticaria, severe AC, and EG/EoD subjects. In these studies, anti-Siglec-8 pharmacodynamic (PD) activity and disease symptom improvements were observed for prolonged periods of time, and the pharmacokinetic (PK) parameters of the antibody demonstrated a long half-life amenable to administration every 4 weeks.

[0279] To date, 51 healthy volunteers (36 on anti-Siglec-8 antibody and 15 on placebo), 25 subjects with ISM, 47 subjects with urticaria (including spontaneous and inducible), 30 subjects with severe AC, 65 subjects with EG/EoD, and 8 subjects with mast cell gastritis/enteritis have been enrolled in clinical studies. In general, the anti-Siglec-8 antibody has been well tolerated. The most common treatment-emergent adverse events (TEAE) observed were mild to moderate infusion-related reactions (IRR) predominantly associated with the first infusion that are thought to be related to ADCC activity of the antibody.

[0280] Subcutaneous (SC) formulations of the antibody have also been developed, as described in the previous Examples. Without wishing to be bound to theory, it is thought that, due to a slower rate of systemic absorption of anti-Siglec-8 antibody when given subcutaneously and the likelihood that maximum plasma concentration (Cmax) will be lower compared to a dose with comparable area under the serum concentration-time curve (AUC), a reduced rate and severity of administration-related reactions may be observed, compared to when the antibody is given intravenously. The study described in this Example is designed to test the safety, tolerability, and bioavailability of subcutaneous administration of the anti-Siglec-8 antibody described in Examples 1 and 2 in healthy volunteers.

Dose Selection

[0281] The proposed doses for SC administration are:

- 0.3mg/kg (Cohort 1);
- 1mg/kg (Cohort 2);
- 3mg/kg (Cohort 3);
- 5mg/kg (Cohort 4); and
- 300mg (Cohort 8): all subjects receive a total of 2mL (300mg anti-Siglec-8 antibody or placebo) of study drug in 1 injection site regardless of body weight.

[0282] These doses are estimated to provide similar or lower exposure than dose levels safely administered by the IV route in various clinical studies.

[0283] Based on previous dosing experience with anti-Siglec-8 antibody IV, as a comparator for determining bioavailability, the proposed anti-Siglec-8 antibody doses for IV administration are:

- 1 mg/kg infused in 100 mL from an IV bag prepared with study drug (Cohort 5);
- 3 mg/kg infused in 100 mL from an IV bag prepared with study drug (Cohort 6); and

- 3 mg/kg infused in 100 mL from an IV bag prepared with study drug (Cohort 7).

Study Design

[0284] Approximately 58 healthy adult volunteers are enrolled among 8 cohorts, including 3 cohorts of 6 subjects each who will receive IV dosing and 5 cohorts of 8 subjects each who will receive SC dosing (6 active and 2 placebo subjects per cohort assigned in a double-blind, randomized manner).

[0285] Cohorts 1, 2, 3, 4, and 8 receive a single dose of anti-Siglec-8 antibody SC (0.3 mg/kg, 1 mg/kg, 3 mg/kg, 5 mg/kg, and 300 mg respectively) or placebo. Subjects in Cohorts 5, 6, and 7 receive a single dose of anti-Siglec-8 antibody IV (1 mg/kg, 3 mg/kg, and 3 mg/kg respectively). The number of subjects is typical for a PK study comparing SC and IV administration of a monoclonal antibody.

[0286] This is a double-blind (for SC cohorts), Phase 1 safety, tolerability, and PK study. Approximately 58 healthy volunteers are enrolled across 1-2 clinical research sites in the United States. Forty subjects receive anti-Siglec-8 antibody SC or placebo, and 18 subjects receive anti-Siglec-8 antibody IV as described above. This is a single-dose study.

[0287] On Day 1, eligible subjects receive a single dose of anti-Siglec-8 antibody (IV or SC) or placebo (SC cohorts) and remain confined to the clinic for 120 hours of monitoring and observation following the end of the infusion or injection. Blood samples from every subject are collected for analysis of anti-Siglec-8 antibody concentration and Complete Blood Count (CBC) with differential (including an absolute eosinophil count) at various time points during the in-patient stay (predose, 1, 3, 6, 8, 12, 24, 48, 72, 96, and 120 hours). Subjects are discharged from the clinic on Day 6 after the 120-hour blood sample has been drawn. Thereafter, subjects return to the clinic on Days 8, 15, 22, 35, 56, and 85, and blood samples are collected for PD and PK analyses.

Study Objectives and Endpoints

[0288] The primary study objective is to assess the safety, tolerability, and pharmacokinetics of the anti-Siglec-8 antibody SC formulation when administered to healthy volunteers as a single dose.

[0289] The secondary study objectives are: (1) to evaluate the pharmacodynamics of the anti-Siglec-8 antibody SC formulation as measured by changes from baseline in absolute peripheral

blood counts of eosinophils, and (2) to determine the bioavailability of the anti-Siglec-8 antibody SC formulation relative to anti-Siglec-8 antibody IV by analyzing the AUC.

[0290] The primary endpoints are safety and tolerability of anti-Siglec-8 antibody administered subcutaneously, and pharmacokinetics, including bioavailability, of anti-Siglec-8 antibody administered subcutaneously.

[0291] The secondary endpoints are to evaluate the pharmacodynamics of anti-Siglec-8 antibody SC as measured by changes from baseline in absolute peripheral blood counts of eosinophils, and to determine the bioavailability of anti-Siglec-8 antibody SC formulation relative to anti-Siglec-8 antibody IV by analyzing the area under the serum AUC.

Subject Selection Criteria

[0292] **Inclusion Criteria:** Subjects are eligible for the study if the following criteria are met:

- Male or female aged ≥18 and ≤65 years at the time of signing the informed consent form;
- Determined by the Investigator to be in good health as documented by medical history, vital signs, physical examination, laboratory assessments, ECG, and by general observations

Test Product, Dose, and Administration

[0293] Single doses of anti-Siglec-8 antibody IV are administered as a peripheral IV infusion (given over 4 hours). Subcutaneous dosing (anti-Siglec-8 antibody or placebo) comprises 1 or 2 SC injections administered in the front of the thigh with a 27-gauge needle. Maximum volume administered per SC injection site is 2 mL.

Safety, PK, and PD Evaluations

[0294] Safety and tolerability are assessed throughout the study by monitoring and evaluating adverse events, including any administration-related reactions (ARR) resulting from IV or SC administration of study drug. All TEAEs are collected from the start of study drug administration through End of Study (EOS). Severity is assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events Version 5.0 (or most current version). All adverse events are assigned a severity grade and assessed to determine whether they are clinically significant and related to study drug.

[0295] For PD evaluations, the eosinophil counts in peripheral blood are collected as described in the study design.

[0296] For PK evaluations, pharmacokinetic blood samples are obtained predose and at various time points described in the study design.

Results

[0297] Results from this phase 1 study in healthy volunteers indicated 63% bioavailability of the anti-Siglec-8 antibody when administered subcutaneously as described above. Subcutaneous administration of the anti-Siglec-8 antibody led to prolonged peripheral blood eosinophil suppression, as shown in **FIG. 9**. For example subcutaneous administration at a dosing level of 3.0 mg/kg, 5.0 mg/kg, and 300 mg led to blood eosinophil depletion identical to that of intravenous infusions dosed at 1.0 mg/kg and 3.0 mg/kg, with the 1.0 mg/kg subcutaneous dosing also identical at all time points except for day 85.

[0298] In addition, antibody treatment was well tolerated, with no injection site reactions or injection reactions, no treatment-related adverse events, and no serious adverse events, indicating that subcutaneous administration as described above appears suitable for once-monthly dosing.

SEQUENCES

All polypeptide sequences are presented N-terminal to C-terminal unless otherwise noted.

All nucleic acid sequences are presented 5' to 3' unless otherwise noted.

Amino acid sequence of mouse 2E2 heavy chain variable domain

QVQLKESGPGLVAPSQSLSITCTVSGFSLTIYGAHWVRQPPGKGLEWLGVIWAGGSTNY
NSALMSRLSISKDNSKSQVFLKINSLQTDDTALYYCARDGSSPYYYSMEYWGQGTSVT
VSS (SEQ ID NO:1)

Amino acid sequence of 2E2 RHA heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAHWVRQAPGKGLEWVSVIWAGGSTN
YNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGTT
VTVSS (SEQ ID NO:2)

Amino acid sequence of 2E2 RHB heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAVSGFSLTIYGAHWVRQAPGKGLEWLGVIWAGGSTN
YNSALMSRLSISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGTT
VTVSS (SEQ ID NO:3)

Amino acid sequence of 2E2 RHC heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAVSGFSLTIYGAHWVRQAPGKGLEWVSVIWAGGSTN
YNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGTT
VTVSS (SEQ ID NO:4)

Amino acid sequence of 2E2 RHD heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAHWVRQAPGKGLEWLSVIWAGGSTN
YNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGTT
VTVSS (SEQ ID NO:5)

Amino acid sequence of 2E2 RHE heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAHWVRQAPGKGLEWVGVIWAGGST NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGT TVTVSS (SEQ ID NO:6)

Amino acid sequence of 2E2 RHF heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAHWVRQAPGKGLEWVSVIWAGGSTN YNSALMSRLTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGTT VTVSS (SEQ ID NO:7)

Amino acid sequence of 2E2 RHG heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAHWVRQAPGKGLEWVSVIWAGGSTN YNSALMSRFSISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGTT VTVSS (SEQ ID NO:8)

Amino acid sequence of 2E2 RHA2 heavy chain variable domain

QVQLQESGPGLVKPSETLSLTCTVSGGSISIYGAHWIRQPPGKGLEWIGVIWAGGSTNYN SALMSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDGSSPYYYSMEYWGQGTLVTV SS (SEQ ID NO:9)

Amino acid sequence of 2E2 RHB2 heavy chain variable domain

QVQLQESGPGLVKPSETLSLTCTVSGFSLTIYGAHWVRQPPGKGLEWLGVIWAGGSTN YNSALMSRLSISKDNSKNQVSLKLSSVTAADTAVYYCARDGSSPYYYSMEYWGQGTL VTVSS (SEQ ID NO:10)

Amino acid sequence of 2E2 RHE S-G mutant heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAHWVRQAPGKGLEWVGVIWAGGST NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYGMEYWGQGT TVTVSS (SEQ ID NO:11)

Amino acid sequence of 2E2 RHE E-D heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAHWVRQAPGKGLEWVGVIWAGGST NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSMDYWGQGT TVTVSS (SEQ ID NO:12)

Amino acid sequence of 2E2 RHE Y-V heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAHWVRQAPGKGLEWVGVIWAGGST NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSMEVWGQGT TVTVSS (SEQ ID NO:13)

Amino acid sequence of 2E2 RHE triple mutant heavy chain variable domain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAHWVRQAPGKGLEWVGVIWAGGST NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYGMDVWGQG TTVTVSS (SEQ ID NO:14)

Amino acid sequence of mouse 2E2 light chain variable domain

QIILTQSPAIMSASPGEKVSITCSATSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVPVRF SGSGSGTSYSLTISRMEAEDAATYYCQQRSSYPFTFGSGTKLEIK (SEQ ID NO:15)

Amino acid sequence of 2E2 RKA light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF SGSGSGTDFTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:16)

Amino acid sequence of 2E2 RKB light chain variable domain

EIILTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLWIYSTSNLASGVPARF SGSGSGTDYTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:17)

Amino acid sequence of 2E2 RKC light chain variable domain

EIILTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARFS GSGSGTDFTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:18)

Amino acid sequence of 2E2 RKD light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLWIYSTSNLASGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:19)

Amino acid sequence of 2E2 RKE light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGVPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:20)

Amino acid sequence of 2E2 RKF light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDYTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:21)

Amino acid sequence of 2E2 RKG light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWYQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIK (SEQ ID NO:22)

Amino acid sequence of 2E2 RKA F-Y mutant light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSSYPYTFGPGTKLDIK (SEQ ID NO:23)

Amino acid sequence of 2E2 RKF F-Y mutant light chain variable domain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDYTLTISSLEPEDFAVYYCQQRSSYPYTFGPGTKLDIK (SEQ ID NO:24)

Amino acid sequence of HEKA heavy chain and HEKF heavy chain

EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAHWVRQAPGKGLEWVGVIWAGGST
NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ

VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:75)

Amino acid sequence of HEKA light chain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDFTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:76)

Amino acid sequence of HEKF light chain

EIVLTQSPATLSLSPGERATLSCSATSSVSYMHWFQQKPGQAPRLLIYSTSNLASGIPARF
SGSGSGTDYTLTISSLEPEDFAVYYCQQRSSYPFTFGPGTKLDIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:77)

Amino acid sequence of IgG1 heavy chain constant region

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:78)

Amino acid sequence of IgG4 heavy chain constant region

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMHEALHNHYTQKSLSLSLG (SEQ ID NO:79)

Amino acid sequence of Ig kappa light chain constant region

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:80)

Amino acid sequence of murine 2C4 and 2E2 IgG1 heavy chain

QVQLKRASGPGLVAPSQSLSITCTVSGFSLTIYGAHWVRQPPGKGLEWLGVIWAGGSTN
YNSALMSRLSISKDNSKSQVFLKINSLQTDDTALYYCARDGSSPYYYSMEYWGQGTSV
TVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPA
VLESDLYTLSSSVTVPSSPRPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSS
VFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNST
FRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMA
KDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMNTNGSYFVYSKLNVQKSN
WEAGNTFTCSVLHEGLHNHHTEKSLSHSPG (SEQ ID NO:81)

Amino acid sequence of murine 2C4 kappa light chain

EIILTQSPAIMSASPGEKVSITCSATSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAEDAATYYCQQRSSYPFTFGSGTKLEIKADAAPTVSIFPPSSEQ
LTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLT
KDEYERHNSYTCEATHKTSTSPIVKSFNRNEC (SEQ ID NO:82)

Amino acid sequence of murine 2E2 kappa light chain

QIILTQSPAIMSASPGEKVSITCSATSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAEDAATYYCQQRSSYPFTFGSGTKLEIKADAAPTVSIFPPSSEQ
LTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLT
KDEYERHNSYTCEATHKTSTSPIVKSFNRNEC (SEQ ID NO:83)

Amino acid sequence of chimeric 2C4 and 2E2 IgG1 heavy chain

QVQLKRASGPGLVAPSQSLSITCTVSGFSLTIYGAHWVRQPPGKGLEWLGVIWAGGSTN
YNSALMSRLSISKDNSKSQVFLKINSLQTDDTALYYCARDGSSPYYYSMEYWGQGTSV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP

REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:84)

Amino acid sequence of chimeric 2C4 kappa light chain
EIILTQSPAIMSASPGEKVSITCSATSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAEDAATYYCQQRSSYPFTFGSGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:85)

Amino acid sequence of chimeric 2E2 kappa light chain
QIILTQSPAIMSASPGEKVSITCSATSSVSYMHWFQQKPGTSPKLWIYSTSNLASGVPVRF
SGSGSGTSYSLTISRMEAEDAATYYCQQRSSYPFTFGSGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:86)

Amino acid sequence of HEKA IgG4 heavy chain (IgG4 contains a S228P mutation)
EVQLVESGGGLVQPGGSLRLSCAASGFSLTIYGAHWVRQAPGKGLEWVGVIWAGGST
NYNSALMSRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARDGSSPYYYSMEYWGQGT
TVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEF
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPR
EEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRL
TVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG (SEQ ID NO:87)

Amino acid sequence of mouse 1C3 heavy chain variable domain (underlined residues comprise CDRs H1 and H2 according to Chothia numbering)
EVQVVESGGDLVKSGGSLKLSCAASGFPFSSYAMSWVRQTPDKRLEWVAIISSGGSYTY
YSDSVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYCARHETAQAAWFAYWGQGTLV
TVSA (SEQ ID NO:106)

Amino acid sequence of mouse 1H10 heavy chain variable domain(underlined residues comprise CDRs H1 and H2 according to Chothia numbering)
EVQLQQSGAELVRPGASVKLSCTASGFNIKDYYMYWVKQRPEQGLEWIGRIAPEDGDT EYAPKFQGKATVTADTSSNTAYLHLSSLTSEDTAVYYCTTEGNYYGSSILDYWGQGTT LTVSS (SEQ ID NO:107)

Amino acid sequence of mouse 4F11 heavy chain variable domain (underlined residues comprise CDRs H1 and H2 according to Chothia numbering)
QVQLQQSGAELVKPGASVKISCKASGYAFRSSWMNWVKQRPGKGLEWIGQIYPGDDY TNYNGKFKGKVTLTADRSSSTAYMQLSSLTSEDSAVYFCARLGPYGPFADWGQGTLVT VSA (SEQ ID NO:108)

Amino acid sequence of mouse 1C3 light chain variable domain
QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKRWIYDTSKLAYGVP ARFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPPTFGGGTKLEIK (SEQ ID NO:109)

Amino acid sequence of mouse 1H10 light chain variable domain
DIQMTQTTSSLSASLGDRVTISCRASQDITNYLNWYQQKPDGTVKLLIYFTSRLHSGVPS RFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPWTFGGGTKLEIK (SEQ ID NO:110)

Amino acid sequence of mouse 4F11 light chain variable domain
QIVLTQSPAIVSASPGEKVTMTCSASSSVSYMYWYQQRPGSSPRLLIYDTSSLASGVPVR FSGSGSGTSYSLTISRIESEDAANYYCQQWNSDPYTFGGGTKLEIK (SEQ ID NO:111)

CLAIMS

What is claimed is:

1. A liquid formulation comprising: (a) a monoclonal antibody that binds to a human Siglec-8, wherein the antibody is in a concentration of about 70mg/mL to about 210mg/mL; and (b) histidine or sodium acetate in a concentration of about 10mM to about 25mM;

wherein the pH of the liquid formulation is between 5.0 and 6.3; and

wherein the antibody comprises: (1) a heavy chain variable region comprising: an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61; an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62; an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and (1) a light chain variable region comprising: an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64; an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO:66.

2. The formulation of claim 1, wherein the antibody is in a concentration of about 135mg/mL to about 165mg/mL.

3. The formulation of claim 1 or claim 2, wherein the antibody is in a concentration of about 150mg/mL.

4. The formulation of any one of claims 1-3, wherein the formulation comprises L-histidine or L-histidine hydrochloride in a concentration of about 15mM.

5. The formulation of claim 4, wherein the pH of the liquid formulation is 6.0.

6. The formulation of any one of claims 1-3, wherein the formulation comprises sodium acetate in a concentration of about 15mM.

7. The formulation of claim 6, wherein the pH of the liquid formulation is about 5.2 to about 5.8.

8. The formulation of claim 7, wherein the pH of the liquid formulation is 5.5.

9. The formulation of any one of claims 1-8, further comprising sucrose in a concentration of about 5% to about 9%.

10. The formulation of claim 9, comprising sucrose in a concentration of about 5% to about 7.5%.

11. The formulation of claim 10, comprising sucrose in a concentration of about 5%.

12. The formulation of any one of claims 1-8, further comprising trehalose in a concentration of about 4% to about 10%.

13. The formulation of claim 12, comprising trehalose in a concentration of about 5% to about 7.5%.

14. The formulation of claim 13, comprising trehalose in a concentration of 6.6%.

15. The formulation of any one of claims 12-14, wherein the trehalose is trehalose dihydrate.

16. The formulation of any one of claims 1-15, further comprising polysorbate 80 in a concentration of about 0.0225% to about 0.0275% (w/v).

17. The formulation of claim 16, wherein the polysorbate 80 is in a concentration of about 0.025% (w/v).

18. The formulation of claim 1, comprising:

(a) the antibody that binds to a human Siglec-8 in a concentration of 150mg/mL;

(b) 15mM L-histidine or L-histidine hydrochloride;

(c) 175mM trehalose dihydrate; and

(d) 0.025% polysorbate 80 (w/v);

wherein the pH of the liquid formulation is 6.0.

19. The formulation of claim 1, comprising:

(a) the antibody that binds to a human Siglec-8 in a concentration of 150mg/mL;

(b) 15mM sodium acetate;

(c) 175mM trehalose dihydrate; and

(d) 0.025% polysorbate 80 (w/v);

wherein the pH of the liquid formulation is 5.5.

20. The formulation of claim 1, comprising:

(a) the antibody that binds to a human Siglec-8 in a concentration of 150mg/mL;

(b) 15mM L-histidine or L-histidine hydrochloride;

(c) 5% sucrose; and

(d) 0.025% polysorbate 80 (w/v);

wherein the pH of the liquid formulation is 6.0.

21. The formulation of any one of claims 1-20, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6; and a light chain variable region comprising the amino acid sequence of SEQ ID NO:16 or 21.

22. The formulation of any one of claims 1-20, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:16.

23. The formulation of any one of claims 1-20, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:6 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:21.

24. The formulation of any one of claims 1-23, wherein the antibody comprises a heavy chain Fc region comprising a human IgG Fc region.

25. The formulation of claim 24, wherein the human IgG Fc region comprises a human IgG1 Fc region.

26. The formulation of claim 25, wherein the human IgG1 Fc region is non-fucosylated.

27. The formulation of claim 24, wherein the human IgG Fc region comprises a human IgG4 Fc region.

28. The formulation of claim 27, wherein the human IgG4 Fc region comprises the amino acid substitution S228P, wherein the amino acid residues are numbered according to the EU index as in Kabat.

29. The formulation of any one of claims 1-20, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:75; and a light chain comprising the amino acid sequence of SEQ ID NO:76 or 77.

30. The formulation of any one of claims 1-20, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:75 and a light chain comprising the amino acid sequence of SEQ ID NO:76.

31. The formulation of any one of claims 1-20, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:75 and a light chain comprising the amino acid sequence of SEQ ID NO:77.

32. The formulation of any one of claims 1-31, wherein the antibody has been engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity.

33. The formulation of any one of claims 1-32, wherein at least one or two of the heavy chains of the antibody is non-fucosylated.

34. An article of manufacture comprising a container enclosing the formulation of any one of claims 1-33.

35. The article of manufacture of claim 34, wherein the container is a glass vial.

36. The article of manufacture of claim 34 or claim 35, further comprising instructions for administering the formulation subcutaneously.