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(54) Titre : METHODES ET COMPOSITIONS POUR LE TRAITEMENT DE L'URTICAIRE CHRONIQUE
(54) Title: METHODS AND COMPOSITIONS FOR TREATING CHRONIC URTICARIA

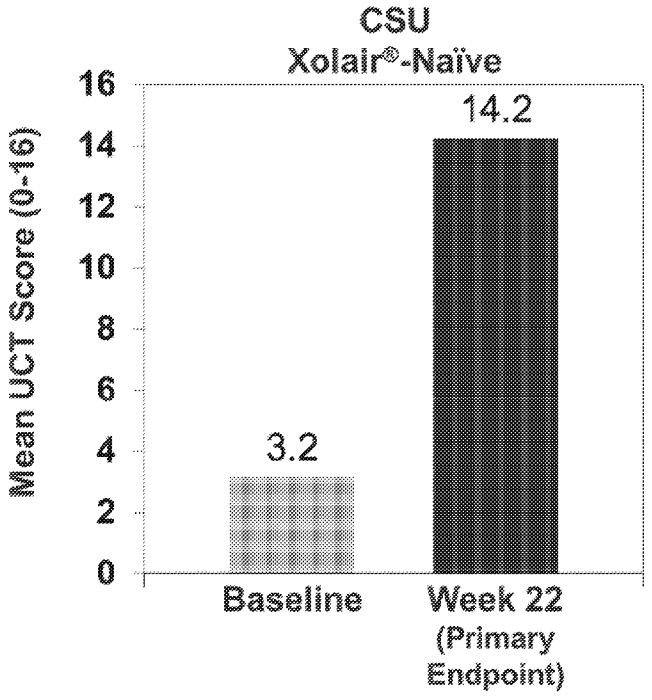

FIG. 1B

(57) Abrégé/Abstract:
The present disclosure provides methods for the treatment of chronic urticaria. In particular, the present disclosure provides methods for the treatment of chronic urticaria through administration of antibodies that bind to human Siglec-8 or compositions comprising said antibodies. The present disclosure also provides articles of manufacture or kits comprising antibodies that bind to human Siglec-8 for the treatment of chronic urticaria.

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(54) Title: METHODS AND COMPOSITIONS FOR TREATING CHRONIC URTICARIA

(57) Abstract: The present disclosure provides methods for the treatment of chronic urticaria. In particular, the present disclosure provides methods for the treatment of chronic urticaria through administration of antibodies that bind to human Siglec-8 or compositions comprising said antibodies. The present disclosure also provides articles of manufacture or kits comprising antibodies that bind to human Siglec-8 for the treatment of chronic urticaria.

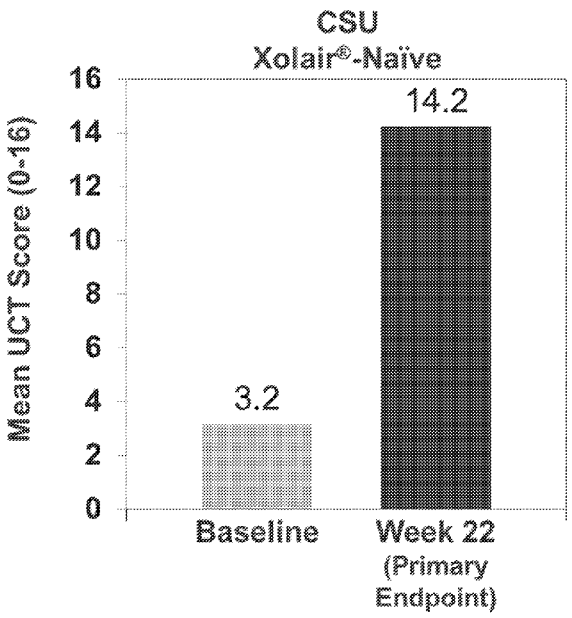

FIG. 1B

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METHODS AND COMPOSITIONS FOR TREATING CHRONIC URTICARIA

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application Serial Nos. 62/667,242, filed May 4, 2018; 62/788,719, filed January 4, 2019; 62/797,817, filed January 28, 2019; 62/803,211, filed February 8, 2019; and 62/806,657, filed February 15, 2019; each 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: 7017112000940SEQLIST.TXT, date recorded: May 2, 2019, size: 123 KB).

FIELD OF THE INVENTION

[0003] The present disclosure relates to methods for treating chronic urticaria by administration of antibodies that bind to human Siglec-8 and compositions comprising said antibodies.

BACKGROUND

[0004] Chronic urticarias are a group of inflammatory skin diseases that are caused by the inappropriate activation of mast cells in the skin. Clinical manifestations include hives (wheals), angioedema or both, which can occur spontaneously or in response to some physical triggers. Chronic urticarias are classified by the specific trigger of mast cell activation. Triggers (and corresponding inducible urticaria types) include: physical skin abrasion (symptomatic dermographism, dermatographic urticaria, or urticaria factitia), pressure, contact, increase in body temperature (cholinergic urticaria), heat, cold (cold-induced urticaria), contact with water (aquagenic), and vibration (vibratory urticaria), with some urticarias triggered by an unknown cause (idiopathic or spontaneous urticaria). *See, e.g.,* Moolani, Y. *et al.* (2016 Feb. 16) *F1000Res.* F1000 Faculty Rev-177. Mast-cell activation in skin lesions of urticaria patients is also enhanced by the infiltration of additional cells into the lesions. In particular, eosinophils are understood to play an important role in chronic urticaria for instance by stimulating the extrinsic coagulation cascade and enhancing mast-cell activity (*see, e.g.,* Asero et al (2009) WAO Journal 2:213-217).

[0005] Many urticaria patients are adequately treated by current therapies such as high dose antihistamines. The most common form of chronic urticaria is chronic spontaneous or idiopathic urticaria. This subtype is believed to be an allergic disease in which IgE plays an important role. Omalizumab (sold as XOLAIR®) is an anti-IgE antibody approved for use in chronic spontaneous urticaria that cannot be managed with H1-antihistamines alone, even at higher than approved doses. However, some patients have demonstrated resistance to omalizumab (*see, e.g.*, Metz, M. *et al.* (2014) *J. Dermatol. Sci.* 73:57-62). The response rate to omalizumab in chronic spontaneous urticaria ranges from 55% to 70-80% when higher than approved doses are used. In the case of physical urticarias, the response rates are lower. In particular, Metz *et al.* described that the response rate to omalizumab in patients with cholinergic urticaria was low, with only 5/8 patients with cholinergic urticaria showing a complete response to omalizumab (as compared to 25/30 for chronic spontaneous urticaria).

[0006] Currently, there is no biologic treatment approved for use in inducible urticarias. A significant number of patients do not receive adequate benefit from current therapies such as H1-antihistamine and/or anti-IgE treatment, and their uncontrolled urticaria symptoms can have a significant impact on quality of life. As such, there remains a need for novel therapeutic approaches that target the inflammation underlying chronic urticarias.

[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 methods of treating or preventing chronic urticaria by administration of antibodies that bind to human Siglec-8 and/or compositions comprising said antibodies. In particular, and due in part to their unique mechanism of action, the methods and compositions described herein may find use in treating individuals that are resistant or refractory to treatment with H1-antihistamine and/or anti-IgE antibody, those with urticaria that is not adequately controlled or urticaria symptoms that remains despite treatment with H1-antihistamine, those that have relapsed after treatment with an anti-IgE antibody, those that have demonstrated an inadequate response to treatment with an anti-IgE antibody, or those whose urticaria symptoms are inadequately controlled by treatment with an anti-IgE antibody. Without wishing to be bound to theory, it is thought that anti-Siglec-8 antibody can inhibit mast cell responses to autoantibodies, *e.g.*, in patients resistant

to anti-IgE antibodies such as omalizumab. In autoimmune urticaria, affected patients produced IgG auto-antibodies against FceRI, IgE, or both, which can cross-link IgE-occupied or unoccupied FceRI to activate mast cells in the skin (*see, e.g.*, Konstantinou, G.N. *et al.* (2013) *Allergy* 68:27-36 and Chang, T.W. *et al.* (2015) *J. Allergy Clin. Immunol.* 135:337-342).

[0009] Accordingly, certain aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of a composition comprising an antibody that binds to human Siglec-8, wherein the individual is resistant or refractory to treatment with H1-antihistamine. Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of an antibody that binds to human Siglec-8, wherein the individual is resistant or refractory to treatment with H1-antihistamine. Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of an antibody that binds to human Siglec-8, wherein the urticaria remains uncontrolled despite treatment with H1-antihistamine. Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of a composition comprising an antibody that binds to human Siglec-8; wherein, prior to administration of the composition, the urticaria in the individual is uncontrolled despite treatment with H1-antihistamine. Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of a composition comprising an antibody that binds to human Siglec-8; wherein, prior to administration of the composition, the urticaria in the individual is not adequately controlled despite treatment with H1-antihistamine. Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of a composition comprising an antibody that binds to human Siglec-8; wherein, prior to administration of the composition, urticaria symptoms in the individual remain despite treatment with H1-antihistamine. Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of a composition comprising an antibody that binds to human Siglec-8; wherein, prior to administration of the composition, the individual remains symptomatic despite treatment with H1-antihistamine.

[0010] Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of a composition comprising an antibody that binds to human Siglec-8, wherein the individual is resistant or refractory to treatment with an anti-IgE antibody or has relapsed after treatment with an anti-IgE antibody. Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of an antibody that binds to human Siglec-8, wherein the individual is resistant or refractory to treatment with an anti-IgE antibody or has relapsed after treatment with an anti-IgE antibody. Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of an antibody that binds to human Siglec-8, wherein the individual has demonstrated an inadequate response to treatment with an anti-IgE antibody or the urticaria is inadequately controlled by treatment with an anti-IgE antibody. Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of a composition comprising an antibody that binds to human Siglec-8; wherein, prior to administration of the composition, the individual has demonstrated an inadequate response to treatment with an anti-IgE antibody or the urticaria in the individual is inadequately controlled by treatment with an anti-IgE antibody. Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of a composition comprising an antibody that binds to human Siglec-8; wherein, prior to administration of the composition, the urticaria in the individual is not adequately controlled despite treatment with an anti-IgE antibody. Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of a composition comprising an antibody that binds to human Siglec-8; wherein, prior to administration of the composition, urticaria symptoms in the individual remain despite treatment with an anti-IgE antibody. Other aspects of the present disclosure relate to methods for treating chronic urticaria in an individual comprising administering to the individual an effective amount of a composition comprising an antibody that binds to human Siglec-8; wherein, prior to administration of the composition, the individual remains symptomatic despite treatment with an anti-IgE antibody.

[0011] Other aspects of the present disclosure relate to methods for decreasing UAS7 score in an individual having chronic urticaria, comprising administering to the individual an effective

amount of a composition comprising an antibody that binds to human Siglec-8; wherein administration of the composition results in a decrease in UAS7 score by 10 or greater, *e.g.*, as compared to a baseline UAS7 score in the individual prior to treatment. In some embodiments, prior to administration of the composition, the individual remains symptomatic or has urticaria that is inadequately controlled despite treatment with H1-antihistamine (*e.g.*, at single or up to four-fold dosage). In some embodiments, prior to administration of the composition, the individual remains symptomatic or has urticaria that is inadequately controlled despite treatment with anti-IgE antibody.

[0012] In some embodiments, the individual is resistant or refractory to treatment with an anti-IgE antibody. In some embodiments, the individual has relapsed after treatment with an anti-IgE antibody. In some embodiments, the individual has demonstrated an inadequate response to treatment with an anti-IgE antibody or the urticaria is inadequately controlled by treatment with an anti-IgE antibody. In some embodiments, prior to administration of the composition, the individual is anti-IgE antibody-naive. In some embodiments, the individual has not been treated with an anti-IgE antibody prior to administration of composition or the antibody that binds to human Siglec-8. In some embodiments, the individual has not been treated with an anti-IgE antibody for at least 2 months prior to administration of composition or the antibody that binds to human Siglec-8. In some embodiments, the individual has not been treated with an anti-IgE antibody for at least 3 months prior to administration of composition or the antibody that binds to human Siglec-8. In some embodiments, the anti-IgE antibody is omalizumab. In some embodiments, the anti-IgE antibody is ligelizumab.

[0013] In some embodiments, the individual is resistant or refractory to treatment with H1-antihistamine. In some embodiments, the individual remains symptomatic despite prior treatment with H1-antihistamine. In some embodiments, prior to administration of the composition, the urticaria in the individual is uncontrolled despite treatment with H1-antihistamine. In some embodiments, the individual is resistant or refractory to treatment with H1-antihistamine at single or label dosage. In some embodiments, the individual remains symptomatic despite prior treatment with H1-antihistamine at single or label dosage. In some embodiments, the individual is resistant or refractory to treatment with H1-antihistamine at four-fold dosage. In some embodiments, the individual remains symptomatic despite treatment with H1-antihistamine at up to four-fold label dosage. In some embodiments, the individual remains symptomatic despite prior treatment with H1-antihistamine at up to four-fold label dosage. In

some embodiments, an individual with uncontrolled urticaria reports a UCT score of less than 12, *e.g.*, despite treatment with H1-antihistamine (*e.g.*, at single or four-fold dosage).

[0014] In some embodiments, the individual is resistant or refractory to treatment with H1-antihistamine and is resistant or refractory to treatment with an anti-IgE antibody. In some embodiments, the individual is resistant or refractory to treatment with H1-antihistamine at single label dosage or up to four-fold label dosage and is resistant or refractory to treatment with an anti-IgE antibody. In some embodiments, the individual remains symptomatic despite prior treatment with H1-antihistamine and treatment with an anti-IgE antibody. In some embodiments, the individual has demonstrated an inadequate response to treatment with an anti-IgE antibody and treatment with H1-antihistamine (*e.g.*, at single or four-fold dosage) or the urticaria is inadequately controlled by treatment with an anti-IgE antibody and treatment with H1-antihistamine (*e.g.*, at single or four-fold dosage).

[0015] In some embodiments, the individual has or has been diagnosed with a chronic urticaria. In some embodiments, the chronic urticaria is a chronic inducible urticaria. In some embodiments, the chronic urticaria is a chronic spontaneous urticaria. In some embodiments, the chronic urticaria is chronic cholinergic, dermatographic or urticaria factitia, cold-induced, vibratory, autoimmune, or idiopathic urticaria. In some embodiments, the chronic urticaria is symptomatic dermographism, chronic cholinergic, dermatographic, heat-induced, aquagenic, solar-induced, pressure- or contact-induced, cold-induced, vibratory, autoimmune, or idiopathic urticaria. In some embodiments, the chronic urticaria is a chronic inducible urticaria of multiple types. In some embodiments, the chronic urticaria is chronic cholinergic urticaria. In some embodiments, the chronic urticaria is chronic dermatographic urticaria or symptomatic dermographism. In some embodiments, the chronic urticaria is chronic autoimmune urticaria, and wherein the individual has demonstrated a positive result in one or more of the following tests before administration of the composition: basophil histamine release assay (BHRA), basophil activation marker expression, autologous serum skin test (ASST), and immunoassay for IgG autoantibodies against IgE and/or FceRI. In some embodiments, the individual has demonstrated a UCT score of less than 12 before administration of the composition. In some embodiments, one or more symptom(s) in the individual with chronic urticaria are reduced as compared to a baseline level before administration of the composition. In some embodiments, self-assessed disease activity is reduced as compared to a baseline level before administration of the composition. In some embodiments, self-assessed disease activity is assessed by one or

more of the following metrics: UCT, UAS7, and CholUAS7. In some embodiments, self-assessed quality-of-life score is improved as compared to a baseline level before administration of the composition. In some embodiments, self-assessed quality-of-life score is assessed by one or more of the following metrics: DLQI, CU-Q2oL, AE-QoL, SD-QoL, and CholU-QoL. In some embodiments, one or more of the following are reduced as compared to a baseline level before administration of the composition: occurrence of angioedema, number of hives, and itch severity. In some embodiments, number of eosinophils, total IgE, expression of tryptase, expression of eosinophil cationic protein, and/or number of basophils in a serum sample from the individual is reduced as compared to a baseline level in a serum sample obtained from the individual before administration of the composition. In some embodiments, administration of the composition results in a sustained response to treatment. In some embodiments, administration of the composition results in a decrease in UAS7 score of greater than 10. In some embodiments, administration of the composition results in a UCT score of 12 or above at 10 weeks after treatment. In some embodiments, administration of the composition results in a UCT score of 12 or above, 13 or above, or 14 or above at week 22 of treatment. In some embodiments, administration of the composition results in a mean increase in UCT score of about 11, or a mean increase in UCT score of about 400%. In some embodiments, administration of the composition results in a complete response to treatment. In some embodiments, a complete response to treatment refers to an improvement of 3 or more in UCT score and a UCT score of greater than 12. In some embodiments, administration of the composition results in a partial response to treatment. In some embodiments, a partial response to treatment refers to an improvement of 3 or more in UCT score. In some embodiments, the composition is administered by intravenous infusion. In some embodiments, the composition is administered by intravenous infusion once a month for 3 or more months. In some embodiments, the composition is administered by subcutaneous injection. In some embodiments, the composition is administered by intravenous infusion at one or more doses comprising between about 0.3 mg/kg and about 3.0 mg/kg of the antibody. In some embodiments, two or more doses comprising between about 0.3 mg/kg and about 3.0 mg/kg of the antibody are administered to the individual at an interval of about 28 days, about 4 weeks, or monthly. In some embodiments, the method comprises administering to the individual a first dose comprising about 0.3 mg/kg of the antibody, a second dose comprising about 1.0 mg/kg of the antibody, a third dose comprising about 1.0 mg/kg of the antibody, a fourth dose comprising

about 1.0 mg/kg to about 3.0 mg/kg of the antibody, a fifth dose comprising about 1.0 mg/kg to about 3.0 mg/kg of the antibody, and a sixth dose comprising about 1.0 mg/kg to about 3.0 mg/kg of the antibody. In some embodiments, the first dose is administered at Day 1, wherein the second dose is administered at Day 29, wherein the third dose is administered at Day 57, wherein the fourth dose is administered at Day 85, wherein the fifth dose is administered at Day 113, and wherein the sixth dose is administered at Day 141. In some embodiments, treatment with the antibody that binds to human Siglec-8 leads to a greater reduction in one or more urticaria symptoms (*e.g.*, as measured by UAS7 and/or UCT score), as compared to treatment with an anti-IgE antibody (*e.g.*, omalizumab or ligelizumab). In some embodiments, the individual has a UAS7 score of 16 or greater prior to treatment with an antibody that binds to human Siglec-8, and treatment with an antibody that binds to human Siglec-8 leads to a greater reduction in UAS7 score, as compared to treatment with an anti-IgE antibody (*e.g.*, omalizumab or ligelizumab). In some embodiments, administration of the composition results in a UAS7 score of 4 or below at week 22 of treatment. In some embodiments, administration of the composition results in a mean reduction in UAS7 score of about 14, or a mean reduction in UAS7 score of about 75%.

[0016] In some embodiments, the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein less than 50% of the N-glycoside-linked carbohydrate chains of the antibody in the composition contain a fucose residue. In some embodiments, substantially none of the N-glycoside-linked carbohydrate chains of the antibody in the composition contain a fucose residue. In some embodiments, the antibody comprises 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. In some embodiments, the antibody comprises 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. 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 selected from SEQ ID NO:16 or 21. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:11-14; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:23-24. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:2-14; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16-24. In some embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:2-10; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16-22. In some embodiments, the antibody comprises: (a) heavy chain variable region 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 region 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. In some embodiments, the antibody comprises: (a) heavy chain variable region comprising: (1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:26; (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61; (3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:34; (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62; (5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:38; (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and (7)

an HC-FR4 comprising the amino acid sequence of SEQ ID NOs:45; and/or (b) a light chain variable region comprising: (1) an LC-FR1 comprising the amino acid sequence of SEQ ID NO:48; (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64; (3) an LC-FR2 comprising the amino acid sequence of SEQ ID NO:51; (4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65; (5) an LC-FR3 comprising the amino acid sequence of SEQ ID NO:55; (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. In some embodiments, the antibody comprises: (a) heavy chain variable region comprising: (1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:26; (2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61; (3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:34; (4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62; (5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:38; (6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and (7) an HC-FR4 comprising the amino acid sequence of SEQ ID NOs:45; and/or (b) a light chain variable region comprising: (1) an LC-FR1 comprising the amino acid sequence of SEQ ID NO:48; (2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64; (3) an LC-FR2 comprising the amino acid sequence of SEQ ID NO:51; (4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65; (5) an LC-FR3 comprising the amino acid sequence of SEQ ID NO: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. 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. In some embodiments, the antibody comprises: a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:106; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:109; a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:107; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:110; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:108; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:111. In some embodiments, the antibody binds to a human Siglec-8 and a non-human primate Siglec-8. In some embodiments, the non-human primate is a baboon. In some embodiments, the antibody 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 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 binds to the same epitope as antibody 4F11. In some embodiments, the antibody binds to an epitope in Domain 2 or Domain 3 of human Siglec-8. In some embodiments, Domain 2 comprises the amino acid sequence of SEQ ID NO:113. In some embodiments, the antibody binds to the same epitope as antibody 1C3. In some embodiments, Domain 3 comprises the amino acid sequence of SEQ ID NO:114. In some embodiments, the antibody binds to the same epitope as antibody 1H10. In some embodiments, the antibody binds to an epitope in Domain 1 of human Siglec-8 and competes with antibody 4F11 for binding to Siglec-8. In some embodiments, the antibody does not compete with antibody 2E2 for binding to Siglec-8. In some embodiments, the antibody is not antibody 2E2. In some embodiments, Domain 1 comprises the amino acid sequence of SEQ ID NO:112. In some embodiments, the antibody is a human antibody, a humanized antibody, or a chimeric antibody. 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, 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 depletes blood eosinophils and/or inhibits mast cell activation. In some embodiments, the antibody has been engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity. In some embodiments, the antibody comprises at least one amino acid substitution in the Fc region that improves ADCC activity. In some embodiments, the antibody has reduced fucosylation as compared to a wild type IgG1. In some embodiments, at least one or two of the heavy chains of the antibody is non-fucosylated. 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 selected from SEQ ID NO:76 or 77. In some embodiments, the anti-Siglec-8 antibody that is used in the methods described herein has a human IgG1 and has reduced fucosylation as compared to a wild type human IgG1. In some embodiments, one or two of the heaving chains of the antibody is non-fucosylated. In some embodiments, the antibody is produced in a mammalian cell line having an alpha1,6-fucosyltransferase (Fut8) knockout. In some embodiments, the antibody is produced in a cell line overexpressing beta 1, 4-N-acetylglycosminyltransferase III (GnT-III). In some embodiments, the cell line additionally overexpresses Golgi μ-mannosidase II (ManII). In some embodiments, the cell line is a CHO cell line. In some embodiments, the antibody is a monoclonal antibody.

[0017] In some embodiments, the composition or antibody is administered in combination with one or more additional therapeutic agent(s) for treating or preventing chronic urticaria. In some embodiments, the one or more additional therapeutic agent(s) for treating or preventing chronic urticaria are selected from the group consisting of H-2 receptor antagonists, H1-antihistamines, H2-antihistamines, anti-IgE antibodies, corticosteroids, doxepin, leukotriene receptor antagonists (LTRAs), cyclosporine, and tacrolimus.

[0018] In some embodiments, the individual is a human. In some embodiments, the treatment results in a complete response in the individual after cessation of the treatment. In some embodiments, one or more symptom(s) in the individual with chronic urticaria are reduced after administration of the composition as compared to a baseline level before administration of the composition. In some embodiments, the treatment results in a complete response in the individual after a single administration of the composition. In some embodiments, the treatment results in at least a 3-point improvement in UCT score in the individual, as compared to UCT score in the individual prior to treatment. In some embodiments, the treatment results in a

reduction in UAS7 score of at least 50% in the individual, as compared to UAS7 score in the individual prior to treatment.

[0019] In some embodiments, the composition comprises the antibody and a pharmaceutically acceptable carrier.

[0020] Other aspects of the present disclosure relate to articles of manufacture or kits comprising a medicament comprising a composition comprising an antibody that binds to human Siglec-8 and a package insert comprising instructions for administration of the medicament in an individual in need thereof according to any one of the above embodiments. Other aspects of the present disclosure relate to articles of manufacture or kits comprising a medicament comprising an antibody that binds to human Siglec-8 and a package insert comprising instructions for administration of the medicament in an individual in need thereof according to any one of the above embodiments.

[0021] Other aspects of the present disclosure relate to a composition comprising an antibody that binds to human Siglec-8 for use in a method of treating chronic urticaria in an individual according to any one of the above embodiments. 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 selected from SEQ ID NO:16 or 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 selected from SEQ ID NO:76 or 77. In some embodiments, at least one or two of the heavy chains of the antibody is non-fucosylated.

[0022] 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

[0023] **FIG. 1A** illustrates the UCT and UAS7 measurement tools for assessing urticaria symptoms. Adapted from EAACI Urticaria guidelines as published in Zuberbier *et al.* (2018) *Allergy* 73:1393-1414.

[0024] **FIG. 1B** shows the effect of anti-Siglec-8 antibody treatment on mean UCT score in anti-IgE-naïve patients with chronic urticaria.

[0025] **FIG. 2A** shows the effect of anti-Siglec-8 antibody treatment on mean UAS7 score in anti-IgE-naïve patients with chronic urticaria.

[0026] **FIG. 2B** shows the proportion of anti-IgE-naïve patients reporting UAS7≤6, or UAS7=0, at week 22.

[0027] **FIG. 2C** shows the proportion of anti-IgE-naïve patients reporting weekly Hive Severity Score (HSS) of 0, or weekly Itch Severity Score (ISS) of 0, at week 22.

[0028] **FIG. 3** shows the effect of anti-Siglec-8 antibody treatment on UAS7 score (change from baseline) in anti-IgE (XOLAIR®, also known as omalizumab)-refractory patients with chronic urticaria.

DETAILED DESCRIPTION

I. Definitions

[0029] 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.

[0030] 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.

[0031] It is understood that aspects and embodiments of the present disclosure include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

[0032] 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.

[0033] 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.

[0034] 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.

[0035] 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.

[0036] 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.

[0037] The term "naked antibody" refers to an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

[0038] 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.

[0039] 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.

[0040] 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.

[0041] 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.

[0042] "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.

[0043] "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).

[0044] "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.

[0045] 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."

[0046] "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.

[0047] 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.

[0048] 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).

[0049] 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

[0050] Unless otherwise indicated, the variable-domain residues (HVR residues and framework region residues) are numbered according to Kabat *et al., supra.*

[0051] "Framework" or "FR" residues are those variable-domain residues other than the HVR residues as herein defined.

[0052] 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.

[0053] 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.

[0054] "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.

[0055] 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).

[0056] 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.

[0057] 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)

[0058] 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.

[0059] 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.

[0060] "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.

[0061] 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.

[0062] "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.

[0063] 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 (1,6)-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.

[0064] 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%).

[0065] "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.

[0066] "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).

[0067] 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.

[0068] 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.

[0069] "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0070] 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 (*e.g.*, chronic urticaria) 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.

[0071] 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.

[0072] 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 (*e.g.*, chronic urticaria).

[0073] As used herein, an individual "at risk" of developing a disease (*e.g.*, chronic urticaria) 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 (*e.g.*, chronic urticaria), 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.

[0074] An "effective amount" refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired or indicated effect, including a therapeutic or prophylactic result. An effective amount can be provided in one or more administrations. A "therapeutically effective amount" is at least the minimum concentration required to effect a measurable improvement of a particular disease. A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. A therapeutically effective amount may also be one in which any toxic or detrimental effects of the antibody are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at the dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in individuals prior to or at the earlier stage of disease, the prophylactically effective amount can be less than the therapeutically effective amount.

[0075] "Chronic" administration refers to administration of the medicament(s) in a continuous as opposed to acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

[0076] 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.

[0077] 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. Methods

[0078] Provided herein are methods for treating and/or preventing chronic urticaria in an individual comprising administering to the individual an effective amount of an antibody described herein that binds to human Siglec-8 (*e.g.*, an anti-Siglec-8 antibody) or compositions comprising said antibodies. In some embodiments, the antibody is in a pharmaceutical composition comprising the antibody and a pharmaceutically acceptable carrier. In some embodiments, the individual is a human.

[0079] In some embodiments, the individual is resistant to treatment with H1-antihistamine (*e.g.*, at single label dosage, or up to four-fold label dosage). In some embodiments, the individual is refractory to treatment with H1-antihistamine (*e.g.*, at single label dosage, or up to four-fold label dosage). In some embodiments, the individual is resistant or refractory to treatment with H1-antihistamine (*e.g.*, at single label dosage, or up to four-fold label dosage) and resistant or refractory to treatment with an anti-IgE antibody (or has relapsed after treatment with an anti-IgE antibody). In some embodiments, the individual remains symptomatic despite treatment with H1-antihistamine (*e.g.*, at single label dosage, or up to four-fold label dosage). In some embodiments, prior to administration of the composition, the urticaria in the individual is uncontrolled despite treatment with H1-antihistamine (*e.g.*, at single label dosage, or up to four-fold label dosage). In some embodiments, prior to administration of the composition, the urticaria in the individual is not adequately controlled despite treatment with H1-antihistamine (*e.g.*, at single label dosage, or up to four-fold label dosage). H1-antihistamines refer to compounds that block or inhibit the action of histamine at the H_1 histamine receptor and have been described as antagonists or inverse agonists of the H_1 histamine receptor. In some embodiments, the H1-antihistamine is a first-generation H1-antihistamine. In some embodiments, the H1-antihistamine is a second-generation H1-antihistamine. Exemplary and non-limiting examples of H1-antihistamines include acrivastine, alimemazine, astemizole, azelastine, Benadryl®, bilastine, bromodiphenhydramine, brompheniramine, buclizine,

carbinoxamine, cetirizine (Zyrtec®), chlorodiphenhydramine, chlorphenamine, clemastine, cyclizine, cyproheptadine, desloratidine, dexbrompheniramine, dexchlorpheniramine, dimenhydrinate, dimetidine, diphenhydramine, doxylamine, ebastine (*e.g.*, carebastine), embramine, fexofenadine, hydroxyzine, levocetirizine, loratidine, meclizine, mequitazine, mirtazapine, mizolastine, olopatadine, orphenadrine, oxatomide, phenindamine, pheniramine, phenyltoloxamine, promethazine, pyrilamine, quetiapine, rupatidine, terfenadine, tripelennamine, and triprolidine.

[0080] In some embodiments, the individual is resistant to treatment with an anti-IgE antibody. In some embodiments, the individual is refractory to treatment with an anti-IgE antibody. In some embodiments, the individual is resistant or refractory to treatment with H1-antihistamine and resistant or refractory to treatment with an anti-IgE antibody (or has relapsed after treatment with an anti-IgE antibody). In some embodiments, the individual has demonstrated an inadequate response to treatment with an anti-IgE antibody or has urticaria that is inadequately controlled by treatment with an anti-IgE antibody. In some embodiments, the individual remains symptomatic despite treatment with an anti-IgE antibody. In some embodiments, prior to administration of the composition, the individual has demonstrated an inadequate response to treatment with an anti-IgE antibody. In some embodiments, prior to administration of the composition, the urticaria in the individual is inadequately controlled by treatment with an anti-IgE antibody. In some embodiments, prior to administration of the composition, the urticaria in the individual is not adequately controlled by treatment with an anti-IgE antibody.

[0081] In some embodiments, the individual has relapsed after treatment with an anti-IgE antibody. In some embodiments, relapse refers to the recurrence of hives and/or itching.

[0082] In some embodiments, the individual has not been treated previously with an anti-IgE antibody (*e.g.*, an anti-IgE antibody naïve individual).

[0083] In some embodiments, the anti-IgE antibody is omalizumab. Omalizumab is a humanized IgG1 anti-IgE antibody that inhibits the binding of IgE to the high-affinity IgE receptor (FcεRI). In some embodiments, the anti-IgE antibody comprises a heavy chain and/or light chain sequence as follows (SEQ ID NOs:125 and 126, respectively). In some embodiments, the anti-IgE antibody comprises a heavy chain variable domain sequence from the heavy chain sequence of SEQ ID NO:125 and/or a light chain variable domain sequence from the light chain sequence of SEQ ID NO:126. In some embodiments, the anti-IgE antibody

comprises 1, 2, or 3 CDRs from the heavy chain sequence of SEQ ID NO:125 and/or 1, 2, or 3 CDRs from the light chain sequence of SEQ ID NO:126.

Heavy chain

EVQLVESGGGLVQPGGSLRLSCAVSGYSITSGYSWNWIRQAPGKGLEWVASITYDGST
NYADSVKGRFTISRDDSKNTFYLQMNSLRAEDTAVYYCARGSHYFGHWHFAVWGQGT
LVTVSSGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:125)

Light chain

DIQLTQSPSSLSASVGDRVTITCRASQSVDYDGDSYMNWYQQKPGKAPKLLIYAASYLE
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSHEDPYTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR (SEQ ID NO:126)

[0084] In some embodiments, the anti-IgE antibody is ligelizumab. Ligelizumab (also known as QGE031) is a humanized IgG1 anti-IgE antibody that is thought to possess a greater affinity for IgE than omalizumab (Arm, J.P. *et al.* (2014) *Clin. Exp. Allergy* 44:1371-1385). In some embodiments, the anti-IgE antibody is an anti-IgE antibody described in U.S. Pat. No. 7,531,169. In some embodiments, the anti-IgE antibody comprises one, two, or three CDRs from the heavy chain variable domain sequence of SEQ ID NO:127 and/or one, two, or three CDRs from the light chain variable domain sequence of SEQ ID NO:128. In some embodiments, the anti-IgE antibody comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:127 and/or a light chain variable domain comprising the amino acid sequence of SEQ ID NO:128.

VH domain

QVQLVQSGAEVMKPGSSVKVSCKASGYTFSWYWLEWVRQAPGHGLEWMGEIDPGTF
TTNYNEKFKARVTFTADTSTSTAYMELSSLRSEDTAVYYCARFSHFSGSNYDYFDYWG
QGTLVTVSS (SEQ ID NO:127)

VL domain

EIVMTQSPATLSVSPGERATLSCRASQSIGTNIHWYQQKPGQAPRLLIYYASESISGIPA
RFSGSGSGTEFTLTISSLQSEDFAVYYCQQSWSWPTTFGGGTKVEIK (SEQ ID NO:128)

[0085] In some embodiments, the individual is resistant or refractory to treatment with H1-antihistamine at label dosage (*e.g.*, an approved dosage of H1-antihistamine, optionally indicated

for the treatment of urticaria). In some embodiments, the individual is resistant or refractory to treatment with H1-antihistamine at single dosage. In some embodiments, the individual is resistant or refractory to treatment with H1-antihistamine at four-fold dosage (*e.g.*, four-fold the label or approved dosage of H1-antihistamine, optionally indicated for the treatment of urticaria).

A. Chronic Urticaria

[0086] Certain aspects of the present disclosure relate to individuals with chronic urticaria. Chronic urticarias are a group of inflammatory skin diseases that are caused by the inappropriate activation of mast cells in the skin, resulting in the appearance of hives or wheals on the skin, itching, irritation, and in some cases angioedema.

[0087] Chronic urticarias are classified by the specific trigger of mast cell activation. In some embodiments, the chronic urticaria is chronic cholinergic urticaria. Cholinergic urticaria is triggered by increase in body temperature and/or sweat. In some embodiments, the chronic urticaria is chronic dermatographic urticaria, symptomatic dermographism, or urticaria factitia. Symptomatic dermographism or dermatographic urticaria is triggered by physical abrasion of the skin and is so named because affected individuals can trace letters or numbers in their skin (*e.g.*, by scratching or other abrasion with an object), which then appear as letter/number-shaped wheals on the skin. In some embodiments, the chronic urticaria is chronic cold-induced urticaria (triggered by cold exposure of the skin). In some embodiments, the chronic urticaria is chronic vibratory urticaria (triggered by vibration, friction, and/or repetitive stretching of the skin). In some embodiments, the chronic urticaria is chronic idiopathic urticaria or chronic spontaneous urticaria. Idiopathic urticaria, also called spontaneous urticaria, is triggered by an unknown cause and/or appears spontaneously.

[0088] In some embodiments, the chronic urticaria is chronic autoimmune urticaria (triggered by autoimmune response). Various clinical and laboratory tests have been proposed for diagnosis of autoimmune urticaria. *See, e.g.,* Konstantinou, G.N. *et al.* (2013) *Allergy* 68:27-36. In some embodiments, an individual diagnosed with chronic autoimmune urticaria has demonstrated a positive result in one or more of the following: basophil histamine release assay (BHRA), basophil activation marker expression (*e.g.*, serum CD63 and/or CD203c), autologous serum skin test (ASST), and immunoassay for IgG autoantibodies against IgE and/or FceRI.

[0089] In some embodiments, the individual has chronic cholinergic, dermatographic, cold-induced, vibratory, autoimmune, or idiopathic urticaria. In some embodiments, the individual

has been diagnosed with chronic cholinergic, dermatographic, symptomatic dermographism, cold-induced, vibratory, autoimmune, or idiopathic urticaria. In some embodiments, the individual has or has been diagnosed with chronic cholinergic dermatographic urticaria. In some embodiments, the individual has or has been diagnosed with chronic dermatographic urticaria or symptomatic dermographism. In some embodiments, the urticaria remains uncontrolled, or not adequately controlled, despite treatment with H1-antihistamine at single or four-fold dosage.

B. Response to Treatment

[0090] In some embodiments, administering to an individual as described herein (*e.g.*, an individual having chronic urticaria) an effective amount of a composition of the present disclosure or antibody described herein that binds to human Siglec-8 (*e.g.*, an anti-Siglec-8 antibody) reduces one or more (*e.g.*, one or more, two or more, three or more, four or more, *etc.*) symptoms in the individual, as compared to a baseline level before administration of the antibody.

[0091] Response to treatment in individuals with chronic urticaria can be assessed by various methods. For example, the Urticaria Control Test (UCT) can be used to provide patient-reported outcomes, *e.g.*, to treatment. UCT is a score for symptom control in chronic urticaria. *See, e.g.*, Weller, K. *et al.* (2014) *J. Allergy Clin. Immunol.* 133:1365-1372. Other techniques or metrics for assessing response to treatment suitable for use as described herein include, without limitation, Urticaria Activity Score (UAS) or UAS7 (*see* www.itchingforanswers.ca/docs/UAS7-Questionnaire.pdf); Cholinergic Urticaria Activity Score (CholUAS) or CholUAS7 (*see* Koch, K. *et al.* (2016) *J. Allergy Clin. Immunol.* 138:1483-1485); number of symptom-free days per week; change in quality of life score as assessed by Dermatology Life Quality Index (DLQI; *see* www.bad.org.uk/shared/get-file.ashx?id=1653&itemtype=document), Chronic Urticaria Quality of Life Questionnaire (CU-Q2oL; *see* Baiardini, I. *et al.* (2005) *Allergy* 60:1073-1078), Angioedema Quality of Life Questionnaire (AE-QoL; *see* Weller, K. *et al.* (2016) *Allergy* 71:1203-1209), Symptomatic Dermographism Quality of Life Questionnaire (SD-QoL), or Cholinergic Urticaria Quality-of-Life Questionnaire (CholU-QoL; *see* Ruft, J. *et al.* (2018) *Clin. Exp. Allergy* 48:433-444); change in occurrence of angioedema (*e.g.*, as assessed by Angioedema Activity Score, AAS; *see* moxie-gmbh.de/media/pdf/aas_scoringtemplate_moxie.pdf); change in number of hives; change in Hive Severity Score (HSS); change in itch severity; change in Itch Severity Score (ISS); change in physician assessment; change in trigger threshold; rates of complete response (CR), partial

response (PR), and non-response (NR); changes in serum tryptase, serum eosinophils, total serum IgE, serum basophils, and/or serum eosinophil cationic protein; and rates of relapse, rebound, or sustained treatment effects. Trigger threshold and changes thereto can be assessed by, for example and without limitation, Pulse Controlled Ergometry Test (PCE; *see* Altrichter, S. *et al.* (2014) *J. Dermatol. Sci.* 75:88-93), FricTest®(*see* moxie-gmbh.de/media/pdf/frictest-instructions-for-use.pdf), or TempTest® (*see* skinobs.com/news/en/suppliers/instrumentation-en/temptest-by-ck-to-determine-cold-and-heat-contact-urticaria/) . UAS score can be broken down further into Hive Severity Score (HSS) and Itch Severity Score (ISS), with each being quantified on a scale from 0 (none) to 3 (intense/severe). Weekly HSS7 and ISS7 scores can be calculated by summing the average respective HSS or ISS scores of the preceding 7 days.

[0092] In some embodiments, self-assessed disease activity (*e.g.*, as assessed by any of the exemplary metrics described *supra*) in an individual is reduced as compared to a baseline level before administration of the composition. In some embodiments, self-assessed disease activity is assessed by one or more of the following metrics: UCT, UAS7, and CholUAS7.

[0093] In some embodiments, an individual has demonstrated a UCT score of less than 12 before administration of a composition or antibody of the present disclosure. For example, in some embodiments, an individual has a UCT score of 12 or less, 11 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, 2 or less, or 1 before administration of a composition or antibody of the present disclosure. In some embodiments, an individual has a UCT score of 12 or above (*e.g.*, 12 or above, 13 or above, 14 or above, 15 or above, or 16) after administration of a composition or antibody of the present disclosure, *e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment. In some embodiments, an individual has a UCT score of 12 or less, 11 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, 2 or less, or 1 before administration of a composition or antibody of the present disclosure, and has a UCT score of 10 or above, 11 or above, 12 or above, 13 or above, 14 or above, 15 or above, or 16 after administration of a composition or antibody of the present disclosure (*e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment), including all possible pairwise combinations thereof wherein the UCT score is higher after treatment than before treatment. For example, in some embodiments, treatment with a composition or antibody of the present disclosure increases UCT score of an individual by at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, or 13 (*e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment), as compared to the UCT score of the individual before treatment. In

some embodiments, treatment with a composition or antibody of the present disclosure increases UCT score of an individual by at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% (*e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment), as compared to the UCT score of the individual before treatment. In some embodiments, treatment with a composition or antibody of the present disclosure increases UCT score of an individual by at least 100%, at least 200%, at least 300%, or at least 400% (*e.g.*, at week 22 of treatment), as compared to the UCT score of the individual before treatment. In some embodiments, the individual has a UCT score of 4 or less or about 3 before administration of a composition or antibody of the present disclosure, and has a UCT score of 14 or above after administration of a composition or antibody of the present disclosure (*e.g.*, at week 22 of treatment). In some embodiments, the individual has a UCT score of 6 or less or about 5 before administration of a composition or antibody of the present disclosure, and has a UCT score of 12 or above after administration of a composition or antibody of the present disclosure (*e.g.*, at week 22 of treatment). In some embodiments, the individual has a UCT score of about 6 before administration of a composition or antibody of the present disclosure, and has a UCT score of 9 or above after administration of a composition or antibody of the present disclosure (*e.g.*, at week 22 of treatment).

[0094] In some embodiments, an individual has demonstrated a UAS7 score of greater than 16 before administration of a composition or antibody of the present disclosure. For example, in some embodiments, an individual has a UAS7 score of 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, or 30 or more before administration of a composition or antibody of the present disclosure. In some embodiments, an individual has a UAS7 score of 16 or less, 15 or less, 14 or less, 13 or less, 12 or less, 11 or less, 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 after administration of a composition or antibody of the present disclosure, *e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment. In some embodiments, an individual has a UAS7 score of 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, or 30 or more before administration of a composition or antibody of the present disclosure, and has a UAS7 score of 16 or less, 15 or less, 14 or less, 13 or less, 12 or less, 11 or less, 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 after administration of a composition or antibody of the present disclosure (*e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment), including all possible pairwise combinations thereof wherein the UAS7 score is higher before treatment than after treatment. For example, in some embodiments, treatment with a composition or antibody of the present disclosure decreases UAS7 score of an individual by at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, or 35 (*e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment), as compared to the UAS7 score of the individual before treatment. In some embodiments, treatment with a composition or antibody of the present disclosure decreases UAS7 score of an individual by at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% (*e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment), as compared to the UAS7 score of the individual before treatment. In some embodiments, treatment with a composition or antibody of the present disclosure decreases UAS7 score of an individual by at least 75% (*e.g.*, at week 22 of treatment), as compared to the UAS7 score of the individual before treatment. In some embodiments, treatment with a composition or antibody of the present disclosure decreases UAS7 score of an individual by 10 or greater, as compared to the UAS7 score of the individual before treatment. For an exemplary description of the UAS metric, *see, e.g.*, Mathias, S.D. *et al.* (2012) *Ann. Allergy Asthma Immunol.* 108:20-24. In some embodiments, an individual has a UAS7 score of 17 or more or about 18 before administration of a composition or antibody of the present disclosure, and has a UAS7 score of 4 or less after administration of a composition or antibody of the present disclosure (*e.g.*, at week 22 of treatment).

[0095] In some embodiments, a complete response refers to a UCT score of 12-16 and change of 3 or greater from baseline. In some embodiments, a partial response refers to a change in UCT score of 3 or greater from baseline. In some embodiments, no response refers to a change in UCT score of less than 3 from baseline. In some embodiments, treatment with a composition or antibody of the present disclosure results in a complete response rate greater than 80%, 85%, or 90%, *e.g.*, in anti-IgE antibody (*e.g.*, XOLAIR®, also known as omalizumab)-naïve individuals with chronic spontaneous urticaria. In some embodiments, treatment with a

composition or antibody of the present disclosure results in a response rate greater than 80%, 85%, or 90%, *e.g.*, in anti-IgE antibody (*e.g.*, XOLAIR®, also known as omalizumab)-naïve individuals with chronic spontaneous urticaria. In some embodiments, treatment with a composition or antibody of the present disclosure results in a complete response rate greater than 80%, *e.g.*, in individuals with cholinergic urticaria. In some embodiments, treatment with a composition or antibody of the present disclosure results in a response rate greater than 80%, *e.g.*, in individuals with cholinergic urticaria. In some embodiments, treatment with a composition or antibody of the present disclosure results in a response rate of at least 70%, *e.g.*, in individuals with symptomatic dermographism. In some embodiments, treatment with a composition or antibody of the present disclosure results in a response rate greater than 50%, *e.g.*, in individuals with anti-IgE antibody (*e.g.*, XOLAIR®, also known as omalizumab)-refractory, chronic spontaneous urticaria (*e.g.*, in individuals with chronic spontaneous urticaria wherein the individual has demonstrated an inadequate response to treatment with an anti-IgE antibody or the chronic urticaria is inadequately controlled by treatment with an anti-IgE antibody).

[0096] In some embodiments, treatment with a composition or antibody of the present disclosure results in an HSS and/or ISS score of 0 in an individual with an HSS and/or ISS score of 1-3 prior to treatment. In some embodiments, treatment with a composition or antibody of the present disclosure results in a reduction in trigger threshold in an individual. For example, in some embodiments, treatment with a composition or antibody of the present disclosure results in a negative FricTest® result in an individual with a positive FricTest® prior to treatment. In some embodiments, treatment with a composition or antibody of the present disclosure results in a negative PCE test result in an individual with a positive PCE test prior to treatment.

[0097] In some embodiments, self-assessed quality-of-life score (*e.g.*, as assessed by any of the exemplary metrics described *supra*) in an individual is improved as compared to a baseline level before administration of the composition. In some embodiments, self-assessed quality-of-life score is assessed by one or more of the following metrics: DLQI, CU-Q2oL, AE-QoL, SD-QoL, and CholU-QoL.

[0098] In some embodiments, an individual has demonstrated a DLQI score of greater than 10 before administration of a composition or antibody of the present disclosure. For example, in some embodiments, an individual has a DLQI score of 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or

more, 22 or more, 23 or more, 24 or more, or 25 or more before administration of a composition or antibody of the present disclosure. In some embodiments, an individual has a DLQI score of 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 after administration of a composition or antibody of the present disclosure, *e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment. In some embodiments, an individual has a DLQI score of 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, or 25 or more before administration of a composition or antibody of the present disclosure, and has a DLQI score of 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 after administration of a composition or antibody of the present disclosure (*e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment), including all possible pairwise combinations thereof wherein the DLQI score is higher before treatment than after treatment. For example, in some embodiments, treatment with a composition or antibody of the present disclosure decreases DLQI score of an individual by at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 (*e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment), as compared to the DLQI score of the individual before treatment. In some embodiments, treatment with a composition or antibody of the present disclosure decreases DLQI score of an individual by at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% (*e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment), as compared to the DLQI score of the individual before treatment.

[0099] In some embodiments, an individual has demonstrated a CU-Q2oL score of greater than 0.60, greater than 0.65, greater than 0.70, greater than 0.75, greater than 0.80, greater than 0.85, or greater than 0.90 before administration of a composition or antibody of the present disclosure. In some embodiments, an individual has a CU-Q2oL score of 0.60 or less, 0.55 or less, 0.50 or less, 0.45 or less, 0.40 or less, 0.35 or less, 0.30 or less, 0.25 or less, or 0.20 or less after administration of a composition or antibody of the present disclosure, *e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment. In some embodiments, an individual has a CU-Q2oL score of greater than 0.50, greater than 0.55, greater than 0.60, greater than 0.65, greater than 0.70, greater than 0.75, greater than 0.80, greater than 0.85, or greater than 0.90 before administration of a composition or antibody of the present disclosure, and has a CU-Q2oL score of 0.60 or less,

0.55 or less, 0.50 or less, 0.45 or less, 0.40 or less, 0.35 or less, 0.30 or less, 0.25 or less, or 0.20 or less after administration of a composition or antibody of the present disclosure (*e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment), including all possible pairwise combinations thereof wherein the CU-Q2oL score is higher before treatment than after treatment. For example, in some embodiments, treatment with a composition or antibody of the present disclosure decreases CU-Q2oL score of an individual by at least .05, at least .10, at least .15, at least .20, at least .25, at least .30, at least .35, at least .40, at least 0.45, or at least .50 (*e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment), as compared to the CU-Q2oL score of the individual before treatment. In some embodiments, treatment with a composition or antibody of the present disclosure decreases CU-Q2oL score of an individual by at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% (*e.g.*, at 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks after treatment), as compared to the CU-Q2oL score of the individual before treatment.

[0100] In some embodiments, treatment with a composition or antibody of the present disclosure leads to a reduction in one or more of: occurrence of angioedema, number of hives, and itch severity, *e.g.*, as compared to a baseline level before administration of the composition or antibody.

[0101] In some embodiments, treatment with a composition or antibody of the present disclosure leads to an increase in one or both of: number of symptom-free days per week and trigger threshold, e.g., as compared to a baseline level before administration of the composition or antibody, or as compared to a suitable reference value.

[0102] In some embodiments, response to treatment with a composition or antibody of the present disclosure is assessed by counting the number of eosinophils and/or basophils in a sample (e.g., a serum sample) obtained from the individual. For example, in some embodiments, number of eosinophils and/or number of basophils in a serum sample from the individual is reduced, e.g., as compared to a baseline level in a serum sample obtained from the individual before administration of the composition or antibody, or as compared to a suitable reference value.

[0103] In some embodiments, response to treatment with a composition or antibody of the present disclosure is assessed by expression level of one or more genes or polypeptides in a sample (e.g., a serum sample) obtained from the individual. For example, in some embodiments, tryptase, eosinophil cationic protein, and/or total IgE in a sample (e.g., a serum

sample) from the individual is reduced, *e.g.*, as compared to a baseline level in a serum sample obtained from the individual before administration of the composition or antibody, or as compared to a suitable reference value.

[0104] In some embodiments, administration of a composition or antibody of the present disclosure results in a sustained response to treatment. In some embodiments, administration of a composition or antibody of the present disclosure results in a complete response to treatment (e.g., after cessation of treatment, or after a single dose of the antibody or composition).

[0105] The terms "baseline" or "baseline value" used interchangeably herein can refer to a measurement or characterization of a symptom before the administration of the therapy (*e.g.*, an anti-Siglec-8 antibody) or at the beginning of administration of the therapy. The baseline value can be compared to a reference value in order to determine the reduction or improvement of a symptom of chronic urticaria contemplated herein. A reference value and/or baseline value can be obtained from one individual, from two different individuals or from a group of individuals (*e.g.*, a group of two, three, four, five or more individuals).

[0106] The terms "reference" or "reference value" used interchangeably herein can refer to a measurement or characterization of a value or symptom in an individual without chronic urticaria (or in a group of such individuals). A "reference value" can be an absolute value; a relative value; a value that has an upper and/or lower limit; a range of values; an average value; a median value; a mean value; or a value as compared to a baseline value. Similarly, a "baseline value" can be an absolute value; a relative value; a value that has an upper and/or lower limit; a range of values; an average value; a median value; a mean value; or a value as compared to a reference value. A reference value can be obtained from one individual, from two different individuals or from a group of individuals (*e.g.*, a group of two, three, four, five or more individuals). In some embodiments, a reference value refers to a standard or benchmark value in the field. In some embodiments, a reference value refers to a value calculated *de novo* from one or more individuals (*e.g.*, without chronic urticaria).

C. Administration

[0107] For the prevention or treatment of disease, the appropriate dosage of an active agent, will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the individual's clinical history and response to the agent, and the discretion of the attending physician. The agent is suitably administered to the individual at one time or over a

series of treatments. In some embodiments, an interval between administrations of an anti-Siglec-8 antibody (*e.g.*, an antibody that binds to human Siglec-8) described herein is about one month or longer. In some embodiments, the interval between administrations is about 1 month, about two months, about three months, about four months, about five months, about six months or longer. As used herein, an interval between administrations refers to the time period between one administration of the antibody and the next administration of the antibody. As used herein, an interval of about one month includes four weeks. Accordingly, in some embodiments, the interval between administrations is about four weeks, about five weeks, about six weeks, about seven weeks, about eight weeks, about nine weeks, about ten weeks, about eleven weeks, about twelve weeks, about sixteen weeks, about twenty weeks, about twenty four weeks, or longer. In some embodiments, the treatment includes multiple administrations of the antibody, wherein the interval between administrations may vary. For example, the interval between the first administration and the second administration is about one month, and the intervals between the subsequent administrations are about three months. In some embodiments, the interval between the first administration and the second administration is about one month, the interval between the second administration and the third administration is about two months, and the intervals between the subsequent administrations are about three months. In some embodiments, an anti-Siglec-8 antibody described herein (*e.g.*, an antibody that binds to human Siglec-8) is administered at a flat dose. In some embodiments, an anti-Siglec-8 antibody described herein (*e.g.*, an antibody that binds to human Siglec-8) is administered to an individual at a dosage from about 0.1 mg to about 1800 mg per dose. In some embodiments, the anti-Siglec-8 antibody (*e.g.*, an antibody that binds to human Siglec-8) is administered to an individual at a dosage of about any of 0.1 mg, 0.5 mg, 1 mg, 5 mg , 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, 1000 mg, 1100 mg, 1200 mg, 1300 mg, 1400 mg, 1500 mg, 1600 mg, 1700 mg, and 1800 mg per dose. In some embodiments, an anti-Siglec-8 antibody described herein (*e.g.*, an antibody that binds to human Siglec-8) is administered to an individual at a dosage from about 150 mg to about 450 mg per dose. In some embodiments, the anti-Siglec-8 antibody (*e.g.*, an antibody that binds to human Siglec-8) is administered to an individual at a dosage of about any of 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, and 450 mg per dose. In some embodiments, an anti-Siglec-8 antibody described herein (*e.g.*, an antibody that binds to human Siglec-8) is administered to an individual

at a dosage from about 0.1 mg/kg to about 20 mg/kg per dose. In some embodiments, an anti-Siglec-8 antibody described herein (*e.g.*, an antibody that binds to human Siglec-8) is administered to an individual at a dosage from about 0.01 mg/kg to about 10 mg/kg per dose. In some embodiments, an anti-Siglec-8 antibody described herein (*e.g.*, an antibody that binds to human Siglec-8) is administered to an individual at a dosage from about 0.1 mg/kg to about 10 mg/kg, about 1.0 mg/kg to about 10 mg/kg, or about 0.3mg/kg to about 1.0 mg/kg. In some embodiments, an anti-Siglec-8 antibody described herein is administered to an individual at a dosage of about any of 0.1 mg/kg, 0.3mg/kg, 0.4mg/kg, 0.5 mg/kg, 0.6mg/kg, 0.7mg/kg, 0.8mg/kg, 0.9mg/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. Any of the dosing frequency described above may be used. Any dosing frequency described above may be used in the methods or uses of the compositions described herein. Efficacy of treatment with an antibody described herein (*e.g.*, an antibody that binds to human Siglec-8) can be assessed using any of the methodologies or assays described herein at intervals ranging between every week and every three months. In some embodiments, efficacy of treatment (*e.g.*, reduction or improvement of one or more symptoms) is assessed about every one month, about every two months, about every three months, about every four months, about every five months, about every six months or longer after administration of an antibody that binds to human Siglec-8. In some embodiments, efficacy of treatment (*e.g.*, reduction or improvement of one or more symptoms) is assessed about every one week, about every two weeks, about every three weeks, about every four weeks, about every five weeks, about every six weeks, about every seven weeks, about every eight weeks, about every nine weeks, about every ten weeks, about every eleven weeks, about every twelve weeks, about every sixteen weeks, about every twenty weeks, about every twenty four weeks, or longer.

[0108] In some embodiments, an anti-Siglec-8 antibody described herein (*e.g.*, an antibody that binds to human Siglec-8) is administered to an individual (*e.g.*, by intravenous infusion) at one or more doses comprising between about 0.1 mg/kg and about 4.0 mg/kg of the antibody. In some embodiments, the antibody is administered to an individual by intravenous infusion at one or more doses comprising between about 0.3 mg/kg and about 3.0 mg/kg of the antibody, *e.g.*, at about 0.3 mg/kg antibody, about 0.5 mg/kg antibody, about 1.0 mg/kg antibody, about 1.5 mg/kg antibody, about 2.0 mg/kg antibody, about 2.5 mg/kg antibody, or about 3.0 mg/kg antibody. In some embodiments, the antibody is administered to the individual (*e.g.*, by intravenous infusion)

in two or more doses (*e.g.*, comprising between about 0.3 mg/kg and about 3.0 mg/kg of the antibody) at an interval of about 28 days. In some embodiments, the antibody is administered to the individual (*e.g.*, by intravenous infusion) monthly in two or more doses (*e.g.*, comprising between about 0.3 mg/kg and about 3.0 mg/kg of the antibody). In some embodiments, the antibody is administered to the individual (*e.g.*, by intravenous infusion) in two or more doses (*e.g.*, comprising between about 0.3 mg/kg and about 3.0 mg/kg of the antibody) at an interval of about 4 weeks. In some embodiments, the antibody is administered to the individual (*e.g.*, by intravenous infusion) in two or more doses (*e.g.*, comprising between about 0.3 mg/kg and about 3.0 mg/kg of the antibody) monthly. In some embodiments, the antibody is administered to the individual (*e.g.*, by intravenous infusion) according to the following schedule: Day 1, Day 29, Day 57, Day 85, Day 113, and Day 141. In some embodiments, the antibody is administered to the individual by intravenous infusion at a first dose comprising about 0.3 mg/kg of the antibody, a second dose comprising about 1.0 mg/kg of the antibody, a third dose comprising about 1.0 mg/kg of the antibody, a fourth dose comprising about 1.0 mg/kg to about 3.0 mg/kg of the antibody, a fifth dose comprising about 1.0 mg/kg to about 3.0 mg/kg of the antibody, and a sixth dose comprising about 1.0 mg/kg to about 3.0 mg/kg of the antibody. In some embodiments, the antibody is administered to the individual by intravenous infusion at a first dose comprising about 0.3 mg/kg of the antibody, a second dose comprising about 1.0 mg/kg of the antibody, a third dose comprising about 1.0 mg/kg of the antibody, a fourth dose comprising about 1.0 mg/kg or about 3.0 mg/kg of the antibody, a fifth dose comprising about 1.0 mg/kg or about 3.0 mg/kg of the antibody, and a sixth dose comprising about 1.0 mg/kg or about 3.0 mg/kg of the antibody. In some embodiments, the antibody is administered to the individual by intravenous infusion at a first dose comprising about 0.3 mg/kg of the antibody, a second dose comprising about 1.0 mg/kg of the antibody, a third dose comprising about 1.0 mg/kg of the antibody, a fourth dose comprising about 1.0 mg/kg of the antibody, a fifth dose comprising about 1.0 mg/kg of the antibody, and a sixth dose comprising about 1.0 mg/kg of the antibody. In some embodiments, the antibody is administered to the individual by intravenous infusion according to the following schedule: about 0.3 mg/kg of the antibody on Day 1, about 1.0 mg/kg of the antibody on Day 29, about 1.0 mg/kg of the antibody on Day 57, about 1.0 mg/kg or about 3.0 mg/kg of the antibody on Day 85, about 1.0 mg/kg or about 3.0 mg/kg of the antibody on Day 113, and about 1.0 mg/kg or about 3.0 mg/kg of the antibody on Day 141. In some embodiments, the dose given at Day 85, 113, or 141 is selected based on the following: 1.0

mg/kg antibody if the individual has experienced symptom improvement and/or a UCT score of ≥ 12, or 3.0 mg/kg antibody if the individual has a UCT score of < 12.

[0109] 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. For instance, an antibody that binds to a human Siglec-8 may be co-administered with one or more (*e.g.*, one or more, two or more, three or more, four or more, *etc.*) additional therapeutic agents for treating and/or preventing chronic urticaria. Therapeutic agents contemplated herein include, but are not limited to, H-2 receptor antagonists, H1-antihistamines, H2-antihistamines, anti-IgE antibodies (*e.g.*, omalizumab), corticosteroids, doxepin, leukotriene receptor antagonists (LTRAs), cyclosporine, and tacrolimus. Although the antibodies of the present disclosure may find use, *e.g.*, in treating individuals that are resistant or refractory to (or have relapsed after treatment with) H1-antihistamines and/or anti-IgE antibodies (*e.g.*, omalizumab), H1-antihistamine and/or anti-IgE antibody treatment may in some cases be used in combination with an antibody of the present disclosure, even if treatment with H1-antihistamines and/or anti-IgE antibodies in the absence of anti-Siglec-8 antibody is sub-optimally effective.

[0110] 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.

[0111] Anti-Siglec8 antibodies and/or one or more additional therapeutic agents may be administered via any suitable route of administration known in the art, including, without limitation, by oral administration, sublingual administration, buccal administration, topical administration, rectal administration, via inhalation, transdermal administration, subcutaneous

injection, intradermal injection, intravenous (IV) injection, intra-arterial injection, intramuscular injection, intracardiac injection, intraosseous injection, intraperitoneal injection, transmucosal administration, vaginal administration, intravitreal administration, intra-articular administration, peri-articular administration, local administration, epicutaneous administration, or any combinations thereof.

D. Antibodies

[0112] 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.

[0113] 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.

[0114] 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.

[0115] 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.

[0116] 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.

[0117] 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.

[0118] 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.

[0119] 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 chronic urticaria. In certain embodiments, a composition of the present disclosure is a pharmaceutical formulation for the prevention of chronic urticaria.

[0120] 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.

[0121] 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.

[0122] 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.

[0123] 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.

[0124] 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.

[0125] 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.

[0126] 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.

[0127] 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.

[0128] 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.

[0129] 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.

[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: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.

[0131] 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.

[0132] 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.

[0133] 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.

[0134] 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).

[0135] 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.

[0136] 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)).

[0137] 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)).

[0138] 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)).

[0139] 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)).

[0140] 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)).

[0141] 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.

[0142] 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)).

[0143] 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.

[0144] 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.

[0145] 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.

[0146] 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.

[0147] 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.

[0148] 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.

[0149] 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.

[0150] 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.

[0151] 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 SEQ ID NO:61	VIWAGGSTNYNSALMS SEQ ID NO:62	DGSSPYYYSMEY 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	VIWAGGSTNYNSALMS	DGSSPYYYGMDV

	SEQ ID NO:61	SEQ ID NO:62	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	WVRQAPGKGLEW	RFTISKDNSKNTVY	WGQGTTVTVSS

	PGGSLRLSCAVSGF SLT (SEQ ID NO:27)	VS (SEQ ID NO:31)	LQMNSLRAEDTAV YYCAR (SEQ ID NO:38)	(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 PSETLSLTCTVSGG SIS (SEQ ID NO:28)	WIRQPPGKGLEWI G (SEQ ID NO:35)	RVTISVDTSKNQFS LKLSSVTAADTAV YYCAR (SEQ ID NO:42)	WGQGTLVTVSS (SEQ ID NO:46)
2E2 RHB2	QVQLQESGPGLVK PSETLSLTCTVSGF SLT (SEQ ID NO:29)	WVRQPPGKGLEW LG (SEQ ID NO:36)	RLSISKDNSKNQVS LKLSSVTAADTAV YYCAR (SEQ ID NO:43)	WGQGTLVTVSS (SEQ ID NO:46)
2E2 RHE S-G	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 RHE E-D	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 RHE Y-V	EVQLVESGGGLVQ PGGSLRLSCAASGF SLT	WVRQAPGKGLEW VG (SEQ ID NO:34)	RFTISKDNSKNTVY LQMNSLRAEDTAV YYCAR	WGQGTTVTVSS (SEQ ID NO:45)

	(SEQ ID NO:26)		(SEQ ID NO:38)	
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 (SEQ ID NO:49)	WFQQKPGQAPRLLIY (SEQ ID NO:51)	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKD	EIVLTQSPATLSLSPGERATLSC (SEQ ID NO:48)	WFQQKPGQAPRLWIY (SEQ ID NO:52)	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC (SEQ ID NO:55)	FGPGTKLDIK (SEQ ID NO:60)
RKE	EIVLTQSPATLSLSPGERATLSC (SEQ ID NO:48)	WFQQKPGQAPRLLIY (SEQ ID NO:51)	GVPARFSGSGSGTDFTLTISSLEPEDFAVYYC (SEQ ID NO:57)	FGPGTKLDIK (SEQ ID NO:60)
RKF	EIVLTQSPATLSLSPGERATLSC (SEQ ID NO:48)	WFQQKPGQAPRLLIY (SEQ ID NO:51)	GIPARFSGSGSGTDYTLTISSLEPEDFAVYYC (SEQ ID NO:58)	FGPGTKLDIK (SEQ ID NO:60)
RKG	EIVLTQSPATLSLSPGERATLSC (SEQ ID NO:48)	WYQQKPGQAPRLLIY (SEQ ID NO:53)	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC (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)

[0152] 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.

[0153] 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 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

[0154] 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 ≤ 1μM, ≤ 150 nM, ≤ 100 nM, ≤ 50 nM, ≤ 10 nM, ≤ 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.

[0155] 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.

[0156] 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).

[0157] 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

[0158] 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

[0159] 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

[0160] 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

[0161] 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.

[0162] 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 (*e.g.*, chronic urticaria). 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).

[0163] 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.

[0164] 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

[0165] 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

[0166] 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.

[0167] 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

[0168] 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.

[0169] 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.

[0170] 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.

3. Human Antibodies

[0171] Human anti-Siglec-8 antibodies of the present disclosure can be constructed by combining Fv clone variable domain sequence(s) selected from human-derived phage display libraries with known human constant domain sequences(s). Alternatively, human monoclonal anti-Siglec-8 antibodies of the present disclosure can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).

[0172] It is possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits et al., *Proc. Natl. Acad. Sci.*

USA, 90: 2551 (1993); Jakobovits et al., *Nature*, 362: 255 (1993); Bruggermann et al., *Year in Immunol.*, 7: 33 (1993).

[0173] Gene shuffling can also be used to derive human antibodies from non-human (*e.g.*, rodent) antibodies, where the human antibody has similar affinities and specificities to the starting non-human antibody. According to this method, which is also called "epitope imprinting", either the heavy or light chain variable region of a non-human antibody fragment obtained by phage display techniques as described herein is replaced with a repertoire of human V domain genes, creating a population of non-human chain/human chain scFv or Fab chimeras. Selection with antigen results in isolation of a non-human chain/human chain chimeric scFv or Fab wherein the human chain restores the antigen binding site destroyed upon removal of the corresponding non-human chain in the primary phage display clone, *i.e.*, the epitope governs the choice of the human chain partner. When the process is repeated in order to replace the remaining non-human chain, a human antibody is obtained (see PCT WO 93/06213 published Apr. 1, 1993). Unlike traditional humanization of non-human antibodies by CDR grafting, this technique provides completely human antibodies, which have no FR or CDR residues of non-human origin.

4. Bispecific Antibodies

[0174] Bispecific antibodies are monoclonal antibodies that have binding specificities for at least two different antigens. In certain embodiments, bispecific antibodies are human or humanized antibodies. In certain embodiments, one of the binding specificities is for Siglec-8 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of Siglec-8. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express Siglec-8. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. $F(ab')_2$ bispecific antibodies).

[0175] Methods for making bispecific antibodies are known in the art. See Milstein and Cuello, Nature, 305: 537 (1983),WO 93/08829 published May 13, 1993, and Traunecker et al., EMBO J., 10: 3655 (1991). For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986). Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Heteroconjugate antibodies may be made using any convenient cross-linking method. Suitable cross-linking agents are well known

in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

5. Single-Domain Antibodies

[0176] In some embodiments, an antibody of the present disclosure is a single-domain antibody. A single-domain antibody is a single polypeptide chain comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, Mass.; see, e.g., U.S. Pat. No. 6,248,516 B1). In one embodiment, a single-domain antibody consists of all or a portion of the heavy chain variable domain of an antibody.

6. Antibody Variants

[0177] In some embodiments, amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody may be prepared by introducing appropriate changes into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

[0178] A useful method for identification of certain residues or regions of the antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) Science, 244:1081-1085. Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is

conducted at the target codon or region and the expressed immunoglobulins are screened for the desired activity.

[0179] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme or a polypeptide which increases the serum half-life of the antibody.

[0180] 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, truncated forms of monoclonal antibodies can be made by recombinant techniques.

[0181] 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.

[0182] 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).

[0183] 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.

[0184] 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).

[0185] 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.

[0186] 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).

[0187] 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

[0188] 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)

[0189] 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.

[0190] 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.

[0191] 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.

[0192] 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.

[0193] 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.

[0194] 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

[0195] 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

[0196] 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.

[0197] 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.

[0198] 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.

[0199] 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.

[0200] 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.

[0201] 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.

[0202] 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.

[0203] 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).

[0204] 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.

[0205] 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.

[0206] 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.

[0207] 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

[0208] 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.

[0209] 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.

[0210] 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.

[0211] 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.

[0212] 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.

[0213] 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.

[0214] 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.

[0215] 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.

[0216] 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.

[0217] 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.

[0218] 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).

[0219] 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

[0220] 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.

[0221] 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.

[0222] 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:

[0223] 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

[0224] 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

[0225] 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

[0226] 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.

[0227] 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.

[0228] 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.

[0229] 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).

[0230] 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

[0231] 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.

[0232] 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.

[0233] 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

[0234] 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

[0235] 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

[0236] 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).

[0237] 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

[0238] 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

[0239] 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.

[0240] 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.

[0241] 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).

[0242] 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

[0243] 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).

[0244] 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.

[0245] 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.

[0246] 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.

[0247] 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.

[0248] 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.

[0249] 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.

[0250] 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. Compositions

[0251] In some aspects, also provided herein are compositions (e.g., pharmaceutical compositions) comprising any of the anti-Siglec-8 antibodies described herein (e.g., an antibody that binds to Siglec-8). In some aspects, provided herein is a composition comprising an anti-Siglec-8 antibody described herein, wherein 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, the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein less than about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, or about 15% of the N-glycoside-linked carbohydrate chains contain a fucose residue. In some aspects, provided herein is a composition comprising an anti-Siglec-8 antibody described herein, wherein the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein substantially none of the N-glycoside-linked carbohydrate chains contain a fucose residue.

[0252] 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.

[0253] Buffers can be used to control the pH in a range which optimizes the therapeutic effectiveness, especially if stability is pH dependent. Buffers can be present at concentrations ranging from about 50 mM to about 250 mM. Suitable buffering agents for use with the present

disclosure include both organic and inorganic acids and salts thereof. For example, citrate, phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate. Additionally, buffers may be comprised of histidine and trimethylamine salts such as Tris.

[0254] Preservatives can be added to prevent microbial growth, and are typically present in a range from about 0.2%-1.0% (w/v). Suitable preservatives for use with the present disclosure include octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium halides (e.g., chloride, bromide, iodide), benzethonium chloride; thimerosal, phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol, 3-pentanol, and m-cresol.

[0255] Tonicity agents, sometimes known as "stabilizers" can be present to adjust or maintain the tonicity of liquid in a composition. When used with large, charged biomolecules such as proteins and antibodies, they are often termed "stabilizers" because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter and intra-molecular interactions. Tonicity agents can be present in any amount between about 0.1% to about 25% by weight or between about 1 to about 5% by weight, taking into account the relative amounts of the other ingredients. In some embodiments, tonicity agents include polyhydric sugar alcohols, trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[0256] 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. Such excipients include: polyhydric sugar alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinisitose, myoinisitol, galactose, galactitol, glycerol, cyclitols (e.g., inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α-monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (e.g., xylose, mannose, fructose, glucose; disaccharides (e.g., lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

[0257] 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. Non-ionic surfactants are present in a range of about 0.05 mg/ml to about 1.0 mg/ml or about 0.07 mg/ml to about 0.2 mg/ml. In some embodiments, non-ionic surfactants are present in a range of about 0.001% to about 0.1% w/v or about 0.01% to about 0.1% w/v or about 0.01% to about 0.025% w/v.

[0258] 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.

[0259] 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.

[0260] 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., injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intraarterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means. In some embodiments, a composition or antibody of the present disclosure is administered by intravenous infusion once a month for 3 or more months.

[0261] 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.

IV. Articles of Manufacture or Kits

[0262] In another aspect, an article of manufacture or kit is provided which comprises 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. Thus, in certain embodiments, the article of manufacture or kit comprises instructions for the use of an anti-Siglec-8 antibody that binds to human Siglec-8 in methods for treating and/or preventing chronic urticaria in an individual comprising administering to the individual an effective amount of an anti-Siglec-8 antibody that binds to human Siglec-8. In certain embodiments, the article of manufacture comprises a medicament comprising an antibody that binds to human Siglec-8 and a package insert comprising instructions for administration of the medicament in an individual in need thereof to treat and/or prevent chronic urticaria. In some embodiments, the package insert further indicates that the treatment is effective in reducing one or more symptoms in the individual with chronic urticaria as compared to a baseline level before administration of the medicament. In some embodiments, the individual is diagnosed with chronic urticaria before administration of the medicament comprising the antibody. In certain embodiments, the individual is a human.

[0263] 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.

[0264] 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, intravenous, or other modes of administration for treating and/or preventing chronic urticaria in an individual. 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.

[0265] 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.

[0266] 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.

[0267] In another aspect, an article of manufacture or kit is provided which comprises 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. Thus, in certain embodiments, the article of manufacture or kit comprises instructions for the use of an anti-Siglec-8 antibody that binds to human Siglec-8 in methods for treating or preventing chronic urticaria in an individual comprising administering to the individual an effective amount of an anti-Siglec-8 antibody that binds to human Siglec-8. In certain embodiments, the article of manufacture or kit comprises a medicament comprising an antibody that binds to human Siglec-8 and a package insert comprising instructions for administration of the medicament in an individual in need thereof to treat and/or prevent chronic urticaria.

[0268] The present disclosure also provides an article of manufacture or kit which comprises an anti-Siglec-8 antibody described herein (e.g., an antibody that binds human Siglec-8) in combination with one or more additional medicament (e.g., a second medicament) for treating or preventing chronic urticaria in an individual. The article of manufacture or kit may further comprise instructions for use of the antibody in combination with one or more additional medicament in the methods of the present disclosure. For example, the article of manufacture or kit herein optionally further comprises a container comprising a second medicament, wherein the anti-Siglec-8 antibody is a first medicament, and which article or kit further comprises instructions on the label or package insert for treating the individual with the second medicament, in an effective amount. Thus in certain embodiments, the article of manufacture or kit comprises instructions for the use of an anti-Siglec-8 antibody that binds to human Siglec-8 in combination with one or more additional medicament in methods for treating or preventing chronic urticaria in an individual. In certain embodiments, the article of manufacture or kit

comprises a medicament comprising an antibody that binds to human Siglec-8 (e.g., a first medicament), one or more additional medicament and a package insert comprising instructions for administration of the first medicament in combination with the one or more additional medicament (e.g., a second medicament). In some embodiments, the one or more additional therapeutic agents may include, but are not limited to, H-2 receptor antagonists, H1-antihistamines, H2-antihistamines, anti-IgE antibodies, corticosteroids, doxepin, leukotriene receptor antagonists (LTRAs), cyclosporine, and tacrolimus.

[0269] 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

[0270] 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: Structure of an open-label, pilot study to assess the efficacy and safety of anti-Siglec-8 antibody treatment in patients with antihistamine-resistant, chronic urticaria

[0271] Chronic urticarias are a group of inflammatory skin diseases that are caused by the inappropriate activation of mast cells in the skin. Chronic urticarias are classified by the specific trigger of mast cell activation. Triggers (and corresponding urticaria types) include: physical skin abrasion (dermatographic urticaria) and increase in body temperature (cholinergic urticaria), with some urticarias triggered by an unknown cause (idiopathic or spontaneous urticaria).

[0272] Many urticaria patients are adequately treated by current therapies such as high dose antihistamines. However, a significant number of patients do not receive adequate benefit from current therapies, and their uncontrolled urticaria symptoms can have a significant impact on quality of life. This study is designed to test the safety and efficacy of anti-Siglec-8 antibody treatment in patients with antihistamine-resistant chronic urticaria.

[0273] Anti-Siglec-8 antibody HEKA (non-fucosylated IgG1) is administered to patients as a monthly intravenous infusion at up to 1mg/kg for 3 doses. All enrolled patients receive 3 monthly infusions and are then followed for another 8 weeks. A total of 40 patients are enrolled.

[0274] Alternatively, anti-Siglec-8 antibody HEKA (non-fucosylated IgG1) is administered to patients as an intravenous infusion. Antibody is administered at Days 1, 29, 57, 85, 113, and 141. Subjects are followed for an additional 8 weeks. Primary and secondary objectives are evaluated at weeks 22, 24, and 28. A total of approximately 48 patients are enrolled. Cohorts include approximately 12 patients with cholinergic urticaria, approximately 12 patients with urticaria factitia (UF), approximately 12 patients that are anti-IgE treatment-naïve (*e.g.*, omalizumab), and approximately 12 patients with chronic spontaneous urticaria that did not achieve an adequate response to anti-IgE treatment (*e.g.*, omalizumab).

[0275] Patients with cholinergic urticaria are tested. Patients are administered HEKA (non-fucosylated IgG1) at a dose of 1 mg/kg by intravenous infusion. Urticaria symptoms are assessed by patients, as described in greater detail below. Patients are given a diary in which the number of wheals and the severity of pruritus are recorded daily for up to 4 weeks immediately before the commencement of therapy and following therapy. Primary and secondary outcome measures are described *infra.*

[0276] Patients with chronic spontaneous urticaria (CSU) are also tested. Patients are administered HEKA (non-fucosylated IgG1) at a dose of 0.3 mg/kg by intravenous infusion as described above. Patients are given a diary in which the number of wheals and the severity of pruritus are recorded daily for at least 27 days immediately before the commencement of therapy and following therapy.

[0277] Alternatively, anti-Siglec-8 antibody is administered to patients according to the following dosing schedule: 0.3 mg/kg at week 0 (day 1), 1.0 mg/kg at week 4 (day 29), 1.0 mg/kg at week 8 (day 57), 1.0 or 3.0 mg/kg at week 12 (day 85), 1.0 or 3.0 mg/kg at week 16 (day 113), and 1.0 or 3.0 mg/kg at week 20 (day 141). At weeks 12, 16, and 20, the dose is increased to 3.0 mg/kg if the UCT score is less than 12 and/or at discretion of the investigator, or the 1.0 mg/kg dose is used if the patient has experienced adequate symptom improvement.

[0278] Primary and secondary outcome measures are described *infra.*

[0279] Inclusion criteria include:

(a) age ($\geq$18 and $\leq$85 years old);

(b) body weight <185kg or <125kg;

(c) diagnosis of chronic urticaria for at least 3 months, refractory to antihistamine treatment in single or 4-fold dosage;

(d) uncontrolled chronic urticaria (UCT<12) at enrollment; and

(e) negative pregnancy test (females).

[0280] Exclusion criteria include:

(a) acute urticaria;

(b) concurrent/ongoing treatment with immunosuppressives (*e.g.*, cyclosporine, methotrexate, dapsone, etc.) within 4 weeks or 5 half-lives prior to baseline, whichever is longer);

(c) significant medical condition rendering the patient immunocompromised;

(d) use of omalizumab within the last 3 months, or within the last 2 months;

(e) receipt of intravenous IgG therapy 30 days prior to baseline;

(f) plasmapheresis 30 days prior to baseline;

(g) Use (daily or every other day) of doxepin 14 days prior to baseline;

(h) receipt of inactive or live attenuated vaccine 30 days prior to baseline;

(i) use of H2 antihistamine 7 days before baseline;

(j) intake of leukotriene antagonist within 7 days prior to enrollment;

(k) intake of systemic corticosteroid (*e.g.*, oral or depot) within 14 days prior to enrollment;

(l) positive screening for ova and parasite test at baseline;

(m) positive HIV serology;

(n) treatment of helminthic parasite within 6 months of screening;

(o) positive hepatitis serology at baseline, except for vaccinated patients or patients with past but resolved hepatitis; and

(p) donation or loss of >500mL blood within 56 days prior to administration of study drug or donation of plasma within 7 days prior to administration of drug.

[0281] The primary outcome measure is change in Urticaria Control Test (UCT) after antibody treatment from day 1 (baseline) to week 10 or from day 1 (baseline) to week 22. UCT is a score for symptom control in chronic urticaria.

[0282] Secondary outcome measures include the following:

(a) Change in disease activity as assessed by Urticaria Activity Score (UAS), which is based on self-documented self-evaluation scores summed over 7 consecutive days (UAS7);

(b) Change in disease activity as assessed by Cholinergic Urticaria Activity Score (CholUAS), which is based on self-documented self-evaluation scores summed over 7 consecutive days (CholUAS7);
(c) Change in number of symptom-free days per week, as assessed by patient diary-based score);
(d) Change in quality-of-life scores, as assessed by Dermatology Life Quality Index (DLQI), Chronic Urticaria Quality of Life Questionnaire (CU-Q2oL), Angioedema Quality of Life Questionnaire (AE-QoL), SD-QoL, or Cholinergic Urticaria Quality-of-Life Questionnaire (CholU-QoL);
(e) Change in occurrence of angioedema (if present), as assessed by Angioedema Activity Score (AAS);
(f) Change in number of hives (from UAS7/ChoUAS7);
(g) Change in itch severity (from UAS7/ChoUAS7);
(h) Change in patient or physician global assessment;
(i) Change in trigger threshold, as assessed by Pulse Controlled Ergometry Test (PCE), FricTest®, or TempTest®;
(j) Rates of complete response (CR), partial response (PR), and non-response (NR), based on QoL, UCT, or UAS7/CholUAS7;
(k) Serum levels at baseline and change of serum levels of biomarkers, including tryptase, eosinophils, total IgE, basophils, and eosinophil cationic protein; and
(l) Rates of treated patients with relapse, rebound, or sustained treatment effects.

Example 2: Effect of anti-Siglec-8 antibody treatment on chronic urticaria in anti-IgE-naïve patients with an inadequate response to antihistamine treatment

[0283] Example 1 describes the design of a clinical trial designed to determine the safety and efficacy of anti-Siglec-8 antibody treatment in patients with antihistamine-resistant chronic urticaria. This Example describes results obtained from the study described in Example 1, focusing on the cohort of patients that were naïve to anti-IgE therapy (*e.g.*, omalizumab treatment).

[0284] The study enrolled 45 patients with uncontrolled chronic urticaria with a UCT score less than 12 despite treatment with H1-antihistamines at doses up to 4x the labeled dosage. Patients were enrolled in 4 cohorts depending on the form of urticaria and prior treatment: chronic spontaneous urticaria Xolair naïve (N=13), chronic spontaneous urticaria Xolair failures

(N=11), cholinergic urticaria (N=11), and dermatographic urticaria (N=10). Antihistamine medication was maintained throughout the screening period and study. Baseline symptom scores, including UCT, were collected over a 4-week screening period. Patients with baseline UCT scores of less than 12 were enrolled in the study and treated with up to 6 doses of anti-Siglec-8 antibody given monthly.

[0285] Patients in the Xolair-naïve cohort ranged in age from 30-75 with a median age of 63.0. Patients were treated with anti-Siglec-8 antibody HEKA (non-fucosylated IgG1) according to the following dosing schedule: 0.3 mg/kg at week 0 (day 1), 1.0 mg/kg at week 4 (day 29), 1.0 mg/kg at week 8 (day 57), 1.0 or 3.0 mg/kg at week 12 (day 85), 1.0 or 3.0 mg/kg at week 16 (day 113), and 1.0 or 3.0 mg/kg at week 20 (day 141). Efficacy was evaluated by change in Urticaria Control Test (UCT), as well as change in disease activity as assessed by Urticaria Activity Score (UAS7). The primary endpoint was at week 22, with follow-ups at weeks 24 and 28.

[0286] Patients with baseline UCT scores less than 12, indicative of poorly controlled urticaria, were enrolled in the study. UCT scores range from 0 to 16 (with 0 being most severe and 16 indicating complete disease control). Patients treated in this study all had UCT scores of below 12 at baseline, with a mean score of 3.2 and 85% of patients reporting a score of 6 or less. UAS7 scores range from 0 to 42, with larger scores indicating greater disease severity. Patients treated in this study had a mean UAS7 score of 18.0.

[0287] As shown in **FIGS. 1A & 1B** and Table A1, anti-Siglec-8 antibody treatment led to a dramatic increase in UCT score (reflecting a decrease in disease severity) among anti-IgE-naïve patients. Mean UCT score increased from 3.2 at baseline to 14.2 at week 22.

Table A1. Responses to anti-Siglec-8 treatment from anti-IgE-naïve cohort.

	Baseline	Week 22
Average UCT Score	3.2	14.2
UCT Complete Response	-	12 (92%)
UCT Partial Response	-	0 (0%)
UCT No Response	-	1 (8%)
Average UAS7 Score	17.9	4.0
Patients with UAS7 <=6	0 (0%)	9 (69%)

UCT Complete response was defined as a greater than a 3-point improvement from baseline and a score greater than 12.
UCT Partial response was defined as a greater than a 3-point improvement from baseline.

[0288] Analysis of individual UCT scores further illustrated this dramatic reduction in disease severity. Remarkably, 92% of anti-IgE-naïve patients (12/13) showed a complete response to anti-Siglec-8 antibody treatment.

[0289] As shown in **FIG. 2A**, anti-Siglec-8 antibody treatment also led to a dramatic reduction in UAS7 score (reflecting a decrease in disease severity) among anti-IgE-naïve patients. Mean UAS7 score decreased from 18.5 at baseline to 4.6 at week 22. Compared to baseline, a 75% reduction in UAS7 score was observed upon administration of the final dose at week 22. Anti-Siglec-8 treatment led to symptom control, as shown by the proportion of patients with UAS7 score or less than or equal to 6, or 0, at week 22 (**FIG. 2B**), and as further demonstrated by the proportion of patients with a weekly Hive Severity Score (HSS) of 0 or weekly Itch Severity Score (ISS) of 0 at week 22 (**FIG. 2C**). The responses of anti-IgE-naïve patients to anti-Siglec-8 antibody treatment are summarized in Table A2.

Table A2. Summary of anti-Siglec-8 treatment on responses and symptom control in anti-IgE-naïve patient cohort.

Endpoint	Baseline	Week 22
UCT Complete Response	-	12/13 (92%)
UCT Partial Response	-	0/13 (0%)
UCT No Response	-	1/13 (8%)
Patients with ΔUAS7 ≥10 (MID)	-	9/13 (69%)
Average UAS7 Score	18.5	4.6 (-75%)
Patients with UAS7 ≤ 6	0 (0%)	8/13 (62%)
Patients with UAS7 =0	0 (0%)	7/13 (54%)
Patients with ISS7 =0	0 (0%)	7/13 (54%)
Patients with HSS7 =0	0 (0%)	10/13 (77%)

UCT Complete response was defined as a greater than a 3-point improvement from baseline and a score greater than 12.
UCT Partial response was defined as a greater than a 3-point improvement from baseline but less than 12.

[0290] Anti-Siglec-8 antibody treatment was generally well tolerated. The most common adverse event was mild to moderate infusion-related reactions (flushing, feeling of warmth, headache, nausea, and dizziness), which occurred mostly during the first infusion. IRR's diminished or did not occur on subsequent infusions.

Example 3: Effect of anti-Siglec-8 antibody treatment in patients with cholinergic and symptomatic dermographism whose symptoms are not adequately controlled by antihistamine treatment

[0291] Whereas there is no identified trigger for chronic spontaneous urticaria, other chronic urticarias are caused by triggers such as physical contact with the skin (referred to as symptomatic dermographism or dermatographic urticaria), or passive or active increases in body temperature (cholinergic urticaria). It has been estimated that 0.5 to 1.0% of the U.S. population suffers from a form of chronic urticaria.

[0292] Example 1 describes the design of a clinical trial designed to determine the safety and efficacy of anti-Siglec-8 antibody treatment in patients with antihistamine-resistant chronic urticaria. This Example describes results obtained from the study described in Example 1, focusing on the cohorts of patients with cholinergic or dermatographic (*e.g.*, symptomatic dermographism) urticaria.

[0293] The cholinergic and dermatographic urticaria cohorts enrolled 11 and 10 patients, respectively, with uncontrolled urticaria despite treatment with H^1 antihistamines at doses of up to 4 times the labeled dosage. Antihistamine medication was maintained throughout the screening period and during the study. Baseline symptom scores, as measured by Urticaria Control Test (UCT) were collected over a 4-week screening period. Patients with baseline UCT scores of less than 12, indicative of poorly controlled urticaria, were enrolled in the study and treated with up to 6 doses of anti-Siglec-8 antibody HEKA (non-fucosylated IgG1) given once monthly. Patients received an initial dose of 0.3 mg/kg at baseline, followed by a dose of 1.0 mg/kg on day 28, and then received monthly doses of either 1.0 or 3.0 mg/kg, depending on response, for a total of 6 doses. The primary efficacy endpoint was change from baseline in UCT assessed at week 22, two weeks after the last dose of anti-Siglec-8 antibody.

[0294] Top-line data from the clinical trial are presented in Table B1. The responses of dermatographic urticaria patients to anti-Siglec-8 antibody treatment are summarized in Table B2. The responses of cholinergic urticaria patients to anti-Siglec-8 antibody treatment are summarized in Table B3.

Table B1. Responses to anti-Siglec-8 treatment from cholinergic and dermatographic urticaria cohorts.

Cholinergic Urticaria Cohort	Baseline	Week 22
Average UCT Score	5.4	11.8
UCT Complete Response	-	9/11 (82%)
UCT Partial Response	-	0/11 (0%)
UCT No Response	-	2/11 (18%)
Dermatographic Urticaria Cohort		
Average UCT Score	5.7	9.1
UCT Complete Response	-	4/10 (40%)
UCT Partial Response	-	3/10 (30%)
UCT No Response	-	3/10 (30%)

UCT complete response was defined as a greater than 3-point improvement from baseline and a score of 12 or greater. UCT partial response was defined as a greater than 3-point improvement from baseline but less than 12.

Table B2. Summary of anti-Siglec-8 treatment on responses and symptom control in dermatographic urticaria cohort.

Endpoint	**Baseline**	**Week 22**
UCT Complete Response	-	4/10 (40%)
UCT Partial Response	-	3/10 (30%)
UCT No Response	-	3/10 (30%)
FRIC Test Itch Response	0%	5/10 (50%)
FRIC Test Hives Response (CFT)	0%	4/10 (40%)

Table B3. Summary of anti-Siglec-8 treatment on responses and symptom control in cholinergic urticaria cohort.

Endpoint	Baseline	Week 22
UCT Complete Response	-	9/11 (82%)
UCT Partial Response	-	0/11 (0%)
UCT No Response	-	2/11 (18%)
PCE Exercise Test Response	0%	7/7 (100%)

[0295] These data, combined with the 92% complete response rate observed in Example 2, show that the anti-Siglec-8 antibody was highly active across multiple types of urticaria and could represent a new promising treatment for chronic spontaneous as well as inducible urticarias. These data indicate that anti-Siglec-8 antibody broadly inhibits mast cell driven inflammation, which, along with its proven activity against eosinophils, suggest that anti-Siglec-8 antibody treatment could have broad utility in eosinophilic and mast cell driven diseases.

Example 4: Effect of anti-Siglec-8 antibody treatment in patients with Xolair refractory chronic spontaneous urticaria

[0296] It has been estimated that 0.5 to 1.0 percent of the U.S. population suffers from a form of chronic urticaria. First-line treatment consists of H_1 antihistamine medication; however, a significant number of patients do not receive adequate benefit even at four times the labeled dose. XOLAIR® is the only agent approved for antihistamine-refractory chronic spontaneous urticaria but is not indicated for other forms of chronic urticaria.

[0297] Example 1 describes the design of a clinical trial designed to determine the safety and efficacy of anti-Siglec-8 antibody treatment in patients with antihistamine-resistant chronic urticaria. This Example describes results obtained from the study described in Example 1, focusing on the cohort of patients with chronic spontaneous urticaria that did not achieve an

adequate response to anti-IgE treatment (*e.g.*, XOLAIR®, also known as omalizumab), as well as additional results from the XOLAIR®-naïve, cholinergic urticaria, and symptomatic dermographism cohorts described in Examples 2 and 3.

[0298] The XOLAIR® failure cohort enrolled 11 patients who failed to have an adequate response to prior XOLAIR® treatment. The cohort had received an average of 10 months of XOLAIR® treatment, and their average UCT score on XOLAIR® was 4.0 at doses as high as 600 mg per month. Patients had to discontinue XOLAIR® for at least two months before screening but could continue H_1 antihistamines at doses of up to four times the labeled dosage throughout the screening period and study. Baseline symptom scores, as measured by Urticaria Control Test (UCT) and Urticaria Activity Score (UAS7), were collected over the 4-week screening period. Patients with baseline UCT scores of less than 12, indicative of poorly-controlled urticaria, were enrolled in the study and treated with an initial dose of 0.3 mg/kg at baseline, followed by a dose of 1.0 mg/kg on day 28, and then received monthly doses of either 1.0 or 3.0 mg/kg, depending on response, for up to six doses. The primary efficacy endpoint was change from baseline in UCT assessed at week 22, two weeks after the last dose of anti-Siglec-8 antibody.

[0299] Data from this cohort are presented in Tables C1 and C2.

Table C1. Response to anti-Siglec-8 treatment from XOLAIR® failure cohort.

Xolair Failure Chronic Spontaneous Urticaria Cohort	**Baseline**	**Week 22**
Average UCT Score	3.7	8.5
UCT Complete Response	-	4/11 (36%)
UCT Partial Response	-	2/11 (18%)
UCT No Response	-	5/11 (45%)
Average UAS7	28.7	14.7 (-52%)

Table C2. Summary of anti-Siglec-8 treatment on responses and symptom control in XOLAIR® refractory patient cohort.

Endpoint	Baseline	Week 22
UCT Complete Response	-	4/11 (36%)
UCT Partial Response	-	2/11 (18%)
UCT No Response	-	5/11 (45%)
Patients with ΔUAS7 ≥10 (MID)	-	8/11 (73%)
Average UAS7 Score	28.7	14.7 (-49%)

[0300] Anti-Siglec-8 antibody treatment showed a 55% (6/11) response rate in patients from this cohort, with an average reduction in UAS7 score of 52%. The change in UAS7 score from baseline, observed at week 22 of anti-Siglec-8 antibody treatment, for each patient in this cohort is shown in **FIG. 3**.

[0301] Anti-Siglec-8 antibody HEKA (non-fucosylated IgG1) was generally well tolerated. The most common adverse event was mild to moderate infusion-related reactions (flushing, feeling of warmth, headache, nausea, and dizziness) which occurred mostly during the first infusion.

Example 5: Additional data from open-label, Phase 2 study on the effects of anti-Siglec-8 antibody treatment in patients with uncontrolled chronic urticaria

[0302] This Example provides additional data on the study described in Example 1.

[0303] The study enrolled 45 patients with uncontrolled chronic urticaria with a UCT score less than 12 despite treatment with H_1 antihistamines at doses up to four times the labeled

dosage. Patients had a median age of 44 with a range from 18-75 years. 75% of the patients were female.

[0304] Patients were enrolled in four cohorts depending on the form of urticaria and prior treatment: chronic spontaneous urticaria Xolair naïve (N=13), chronic spontaneous urticaria Xolair failures (N=11), cholinergic urticaria (N=11) and symptomatic dermographism (N=10). Antihistamine medication was maintained throughout the screening period and study. Baseline symptom scores, including UCT, were collected over a 4-week screening period. Patients with baseline UCT scores of less than 12 were enrolled in the study and treated with up to six doses of anti-Siglec-8 antibody HEKA (non-fucosylated IgG1) given monthly.

[0305] Patients received an initial dose of 0.3 mg/kg, 1.0 mg/kg on day 28, and then received monthly doses of either 1.0 or 3.0 mg/kg for a total of six doses. For doses 4, 5, and 6, dose was increased from 1.0 mg/kg to 3.0 mg/kg if the UCT score was less than 12. Efficacy was assessed at week 22 using the urticaria control test (UCT) and UAS7 (for patients with chronic spontaneous urticaria only). UCT Complete response was defined as a greater than a 3-point improvement from baseline and a score greater than 12. UCT Partial response was defined as a greater than a 3-point improvement from baseline.

[0306] Additional data from this study are shown in Table D. Baseline UCT and UAS7 scores from each cohort are shown in Table E.

Table D. Response to anti-Siglec-8 treatment from 3 cohorts of patients with uncontrolled chronic urticaria.

Xolair Naïve CSU Cohort (N=13)	**Baseline**	**Week 22**
Average UCT Score	3.2	14.2
UCT Complete Response	-	12/13 (92%)
UCT Partial Response	-	0/13 (0%)
UCT No Response	-	1/13 (8%)
Average UAS7 Score	18.5	4.6 (-75%)
Proportion with UAS7 ≤ 6	0%	8/13 (62%)
Proportion with UAS = 0	0%	7/13 (54%)
Proportion with ISS = 0	0%	7/13 (54%)
Proportion with HSS = 0	0%	10/13 (77%)
Cholinergic Urticaria Cohort (N=11)	**Baseline**	**Week 22**
Average UCT Score	5.4	11.8
UCT Complete Response	-	9/11 (82%)
UCT Partial Response	-	0/11 (0%)
UCT No Response	-	2/11 (18%)
Pulse Control Ergometry (PCE) Exercise Test Negative	0%	7/7 (100%)
Symptomatic Dermographism Cohort (N=10)	**Baseline**	**Week 22**
Average UCT Score	5.7	9.1
UCT Complete Response	-	4/10 (40%)
UCT Partial Response	-	3/10 (30%)
UCT No Response	-	3/10 (30%)
FricTest® Itch Negative	0%	5/10 (50%)
FricTest® Hives Negative (Critical Friction Threshold)	0%	4/10 (40%)

Table E. Baseline mean UCT and UAS7 scores

	XN (n=13)	XF (n=11)	CholU (n=11)	SDerm (n=10)
UCT	3.2	3.7	5.4	5.7
UAS7	18.5	28.7	n/a	n/a

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 method for treating chronic urticaria in an individual comprising administering to the individual an effective amount of a composition comprising an antibody that binds to human Siglec-8; wherein, prior to administration of the composition, the urticaria in the individual is not adequately controlled, or urticaria symptoms remain, despite treatment with H1-antihistamine.

2. The method of claim 1, wherein the individual has demonstrated an inadequate response to treatment with an anti-IgE antibody or the chronic urticaria is inadequately controlled by treatment with an anti-IgE antibody.

3. The method of claim 1, wherein, prior to administration of the composition, the individual is anti-IgE antibody-naive.

4. The method of claim 2 or claim 3, wherein the anti-IgE antibody is omalizumab or ligelizumab.

5. A method for treating chronic urticaria in an individual comprising administering to the individual an effective amount of a composition comprising an antibody that binds to human Siglec-8; wherein, prior to administration of the composition, the individual has demonstrated an inadequate response to treatment with an anti-IgE antibody or the urticaria in the individual is inadequately controlled by treatment with an anti-IgE antibody.

6. The method of claim 5, wherein the anti-IgE antibody is omalizumab or ligelizumab.

7. The method of claim 5 or claim 6, wherein, prior to administration of the composition, the urticaria in the individual is uncontrolled despite treatment with H1-antihistamine.

8. The method of any one of claims 1-4 and 7, wherein, prior to administration of the composition, the urticaria in the individual is uncontrolled despite treatment with H1-antihistamine at label dosage or four-fold label dosage.

9. The method of any one of claims 1-4 and 7, wherein, prior to administration of the composition, the urticaria in the individual is uncontrolled despite treatment with H1-antihistamine at up to four-fold label dosage.

10. The method of any one of claims 1-9, wherein the chronic urticaria is chronic cholinergic, dermatographic, cold-induced, vibratory, autoimmune, spontaneous, or idiopathic urticaria.

11. The method of any one of claims 1-10, wherein the chronic urticaria is chronic autoimmune urticaria, and wherein the individual has demonstrated a positive result in one or more of the following tests before administration of the composition: basophil histamine release assay (BHRA), basophil activation marker expression, autologous serum skin test (ASST), and immunoassay for IgG autoantibodies against IgE and/or FceRI.

12. The method of any one of claims 1-11, wherein the individual has demonstrated a UCT score of less than 12 before administration of the composition.

13. The method of any one of claims 1-12, wherein one or more symptom(s) in the individual with chronic urticaria are reduced after administration of the composition, as compared to a baseline level before administration of the composition.

14. The method of claim 13, wherein self-assessed disease activity is reduced as compared to a baseline level before administration of the composition.

15. The method of claim 14, wherein self-assessed disease activity is assessed by one or more of the following metrics: UCT, UAS7, and CholUAS7.

16. The method of claim 13, wherein self-assessed quality-of-life score is improved as compared to a baseline level before administration of the composition.

17. The method of claim 16, wherein self-assessed quality-of-life score is assessed by one or more of the following metrics: DLQI, CU-Q2oL, AE-QoL, SD-QoL, and CholU-QoL.

18. The method of claim 13, wherein one or more of the following are reduced as compared to a baseline level before administration of the composition: occurrence of angioedema, number of hives, and itch severity.

19. The method of claim 13, wherein one or more of the following are increased as compared to a baseline level before administration of the composition: number of symptom-free days per week and trigger threshold.

20. The method of any one of claims 1-19, wherein number of eosinophils, total IgE, expression of tryptase, expression of eosinophil cationic protein, and/or number of basophils in a serum sample from the individual is reduced as compared to a baseline level in a serum sample obtained from the individual before administration of the composition.

21. The method of any one of claims 1-20, wherein administration of the composition results in a sustained response to treatment.

22. The method of any one of claims 1-20, wherein administration of the composition results in a UCT score of 12 or above at 10 weeks after treatment.

23. The method of any one of claims 1-20, wherein administration of the composition results in a UCT score of 12 or above at 22 weeks after treatment.

24. The method of any one of claims 1-20, wherein administration of the composition results in a decrease in UAS7 score of greater than 10.

25. The method of any one of claims 1-24, wherein the composition is administered by intravenous infusion.

26. The method of claim 25, wherein the composition is administered by intravenous infusion once a month for 3 or more months.

27. The method of any one of claims 1-24, wherein the composition is administered by subcutaneous injection.

28. The method of any one of claims 1-24, wherein the composition is administered by intravenous infusion at one or more doses comprising between about 0.3 mg/kg and about 3.0 mg/kg of the antibody.

29. The method of claim 28, wherein two or more doses comprising between about 0.3 mg/kg and about 3.0 mg/kg of the antibody are administered to the individual at an interval of about 28 days, about 4 weeks, or monthly.

30. The method of claim 28 or 29, wherein the method comprises administering to the individual a first dose comprising about 0.3 mg/kg of the antibody, a second dose comprising about 1.0 mg/kg of the antibody, a third dose comprising about 1.0 mg/kg of the antibody, a fourth dose comprising about 1.0 mg/kg to about 3.0 mg/kg of the antibody, a fifth dose comprising about 1.0 mg/kg to about 3.0 mg/kg of the antibody, and a sixth dose comprising about 1.0 mg/kg to about 3.0 mg/kg of the antibody.

31. The method of claim 30, wherein the first dose is administered at Day 1, wherein the second dose is administered at Day 29, wherein the third dose is administered at Day 57, wherein the fourth dose is administered at Day 85, wherein the fifth dose is administered at Day 113, and wherein the sixth dose is administered at Day 141.

32. The method of any one of claims 1-31, wherein the antibody comprises a Fc region and N-glycoside-linked carbohydrate chains linked to the Fc region, wherein less than 50% of the N-glycoside-linked carbohydrate chains of the antibody in the composition contain a fucose residue.

33. The method of claim 32, wherein substantially none of the N-glycoside-linked carbohydrate chains of the antibody in the composition contain a fucose residue.

34. The method of any one of claims 1-33, wherein the antibody comprises 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.

35. The method of any one of claims 1-33, wherein the antibody comprises 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.

36. The method of any one of claims 1-33, wherein 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 selected from SEQ ID NO:16 or 21.

37. The method of any one of claims 1-33, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:11-14; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:23-24.

38. The method of any one of claims 1-33, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:2-14; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16-24.

39. The method of any one of claims 1-33, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence selected from SEQ ID NOs:2-10; and/or a light chain variable region comprising the amino acid sequence selected from SEQ ID NOs:16-22.

40. The method of any one of claims 1-33, wherein the antibody comprises:

(a) heavy chain variable region 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 region 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.

41. The method of any one of claims 1-33, wherein the antibody comprises:

(a) heavy chain variable region comprising:

(1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:26;

(2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61;

(3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:34;

(4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62;

(5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:38;

(6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and

(7) an HC-FR4 comprising the amino acid sequence of SEQ ID NOs:45; and/or

(b) a light chain variable region comprising:

(1) an LC-FR1 comprising the amino acid sequence of SEQ ID NO:48;

(2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64;

(3) an LC-FR2 comprising the amino acid sequence of SEQ ID NO:51;

(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65;

(5) an LC-FR3 comprising the amino acid sequence of SEQ ID NO:55;

(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.

42. The method of any one of claims 1-33, wherein the antibody comprises:

(a) heavy chain variable region comprising:

(1) an HC-FR1 comprising the amino acid sequence of SEQ ID NO:26;

(2) an HVR-H1 comprising the amino acid sequence of SEQ ID NO:61;

(3) an HC-FR2 comprising the amino acid sequence of SEQ ID NO:34;

(4) an HVR-H2 comprising the amino acid sequence of SEQ ID NO:62;

(5) an HC-FR3 comprising the amino acid sequence of SEQ ID NO:38;

(6) an HVR-H3 comprising the amino acid sequence of SEQ ID NO:63; and

(7) an HC-FR4 comprising the amino acid sequence of SEQ ID NOs:45; and/or

(b) a light chain variable region comprising:

(1) an LC-FR1 comprising the amino acid sequence of SEQ ID NO:48;

(2) an HVR-L1 comprising the amino acid sequence of SEQ ID NO:64;

(3) an LC-FR2 comprising the amino acid sequence of SEQ ID NO:51;

(4) an HVR-L2 comprising the amino acid sequence of SEQ ID NO:65;

(5) an LC-FR3 comprising the amino acid sequence of SEQ ID NO: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.

43. The method of any one of claims 1-33, wherein 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.

44. The method of any one of claims 1-33, wherein the antibody comprises:

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:106; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:109;

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:107; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:110; or

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:108; and/or a light chain variable region comprising the amino acid sequence of SEQ ID NO:111.

45. The method of any one of claims 1-33, wherein the antibody binds to a human Siglec-8 and a non-human primate Siglec-8.

46. The method of claim 45, wherein the non-human primate is a baboon.

47. The method of claim 45, wherein the antibody binds to an epitope in Domain 1 of human Siglec-8, wherein Domain 1 comprises the amino acid sequence of SEQ ID NO:112.

48. The method of claim 45, wherein the antibody binds to an epitope in Domain 3 of human Siglec-8, wherein Domain 3 comprises the amino acid sequence of SEQ ID NO:114.

49. The method of claim 45, wherein the antibody binds to the same epitope as antibody 4F11.

50. The method of any one of claims 1-33, wherein the antibody binds to an epitope in Domain 2 or Domain 3 of human Siglec-8.

51. The method of claim 50, wherein Domain 2 comprises the amino acid sequence of SEQ ID NO:113.

52. The method of claim 50, wherein the antibody binds to the same epitope as antibody 1C3.

53. The method of claim 50, wherein Domain 3 comprises the amino acid sequence of SEQ ID NO:114.

54. The method of claim 50, wherein the antibody binds to the same epitope as antibody 1H10.

55. The method of any one of claims 1-33, wherein the antibody binds to an epitope in Domain 1 of human Siglec-8 and competes with antibody 4F11 for binding to Siglec-8.

56. The method of claim 55, wherein the antibody does not compete with antibody 2E2 for binding to Siglec-8.

57. The method of claim 56, wherein the antibody is not antibody 2E2.

58. The method of claim 55, wherein Domain 1 comprises the amino acid sequence of SEQ ID NO:112.

59. The method of any one of claims 34-58, wherein the antibody is a human antibody, a humanized antibody, or a chimeric antibody.

60. The method of any one of claims 34-59, wherein the antibody depletes blood eosinophils and inhibits mast cell activation.

61. The method of any one of claims 1-60, wherein the antibody comprises a heavy chain Fc region comprising a human IgG Fc region.

62. The method of claim 61, wherein the human IgG Fc region comprises a human IgG1 Fc region.

63. The method of claim 62, wherein the human IgG1 Fc region is non-fucosylated or has reduced fucosylation.

64. The method of claim 61, wherein the human IgG Fc region comprises a human IgG4 Fc region.

65. The method of claim 64, 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.

66. The method of any one of claims 1-58, wherein the antibody has been engineered to improve antibody-dependent cell-mediated cytotoxicity (ADCC) activity.

67. The method of claim 66, wherein the antibody comprises at least one amino acid substitution in the Fc region that improves ADCC activity.

68. The method of any one of claims 1-60, wherein at least one or two of the heavy chains of the antibody is non-fucosylated.

69. The method of any one of claims 1-33, wherein 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 selected from SEQ ID NO:76 or 77.

70. The method of any one of claims 1-69, wherein the antibody is a monoclonal antibody.

71. The method of any one of claims 1-70, wherein the composition is administered in combination with one or more additional therapeutic agent(s) for treating or preventing chronic urticaria.

72. The method of claim 71, wherein the one or more additional therapeutic agent(s) for treating or preventing chronic urticaria are selected from the group consisting of H-2 receptor antagonists, H1-antihistamines, H2-antihistamines, anti-IgE antibodies, corticosteroids, doxepin, leukotriene receptor antagonists (LTRAs), cyclosporine, and tacrolimus.

73. The method of any one of claims 1-72, wherein the individual is a human.

74. The method of any one of claims 1-73, wherein the treatment results in a complete response in the individual after a single administration of the composition.

75. The method of any one of claims 1-73, wherein the treatment results in at least a 3-point improvement in UCT score in the individual, as compared to UCT score in the individual prior to treatment.

76. The method of any one of claims 1-73, wherein the treatment results in a reduction in UAS7 score of at least 50% in the individual, as compared to UAS7 score in the individual prior to treatment.

77. The method of any one of claims 1-73, wherein the treatment results in a reduction in UAS7 score by at least 10 in the individual, as compared to UAS7 score in the individual prior to treatment.

78. The method of any one of claims 1-77, wherein the composition comprises the antibody and a pharmaceutically acceptable carrier.

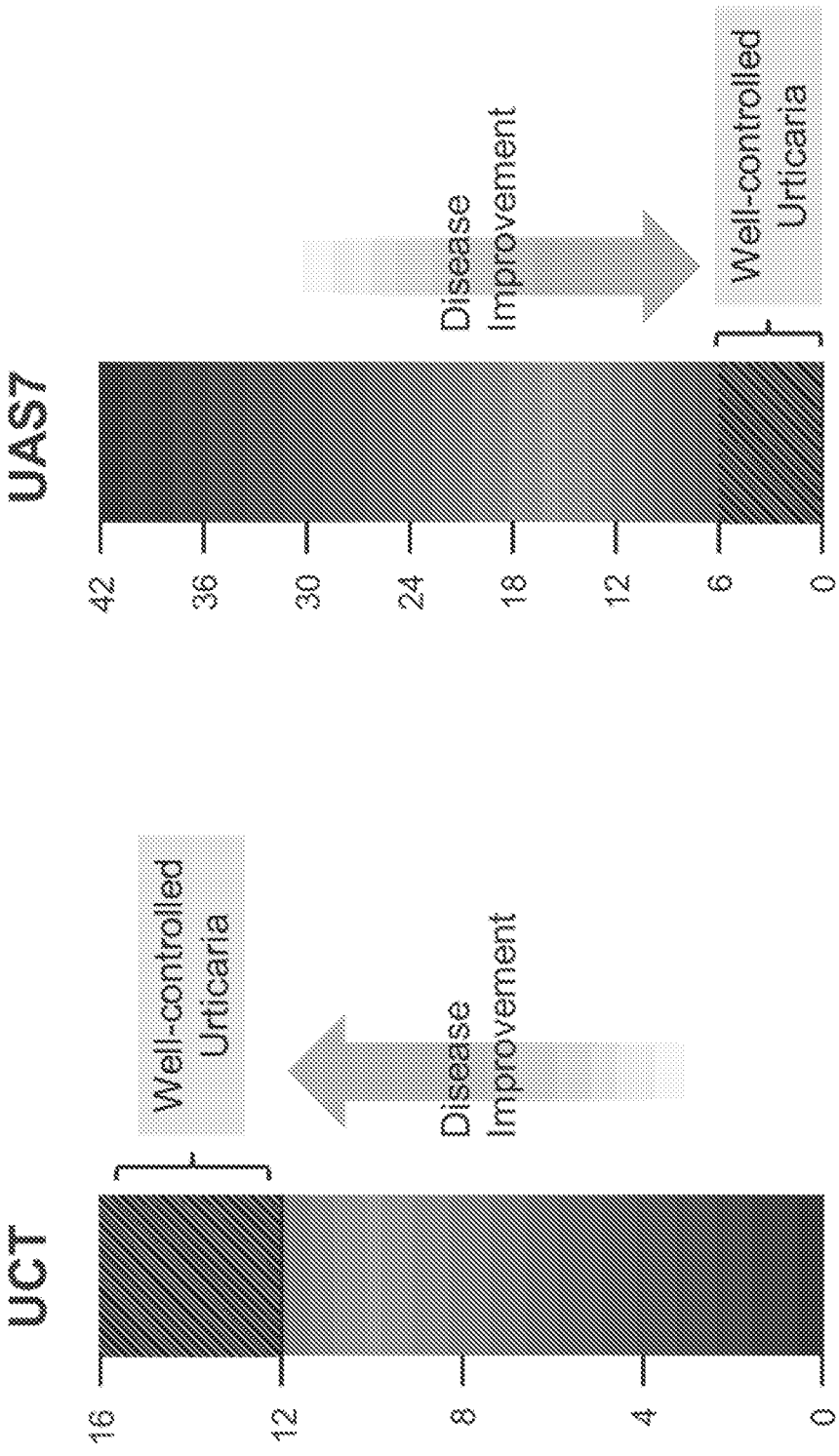
79. An article of manufacture comprising a medicament comprising a composition comprising an antibody that binds to human Siglec-8 and a package insert comprising instructions for administration of the medicament in an individual in need thereof according to any one of claims 1-78.

80. A composition comprising an antibody that binds to human Siglec-8 for use in a method of treating chronic urticaria in an individual according to any one of claims 1-78.

81. The composition for use of claim 80, wherein 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 selected from SEQ ID NO:16 or 21.

82. The composition for use of claim 80, wherein 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 selected from SEQ ID NO:76 or 77.

83. The composition for use of any one of claims 80-82, wherein at least one or two of the heavy chains of the antibody is non-fucosylated.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30
24
18
12
6
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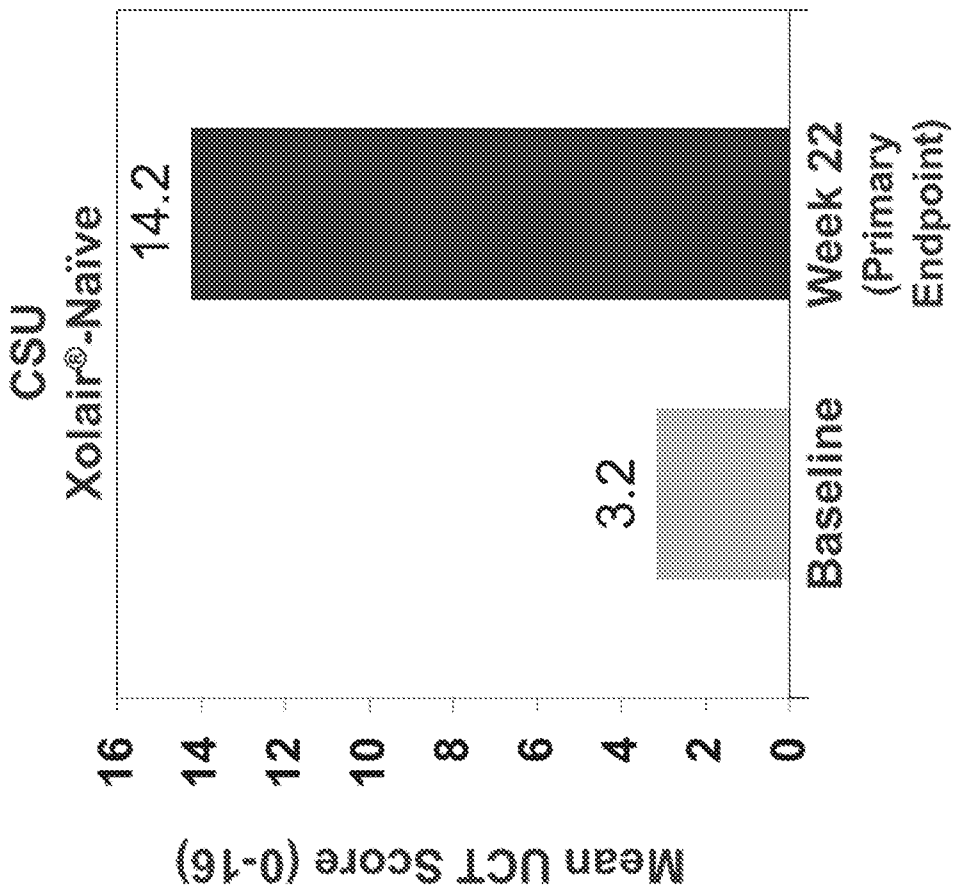
Disease Improvement
Well-controlled Urticaria
UCT
16
12
8
4
0
Well-controlled Urticaria
Disease Improvement

FIG. 1A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Xolair®-Naïve
14.2
3.2
Baseline
Week 22
(Primary
Endpoint)
Mean UCT Score (0-16)
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14
12
10
8
6
4
2
0

FIG. 1B

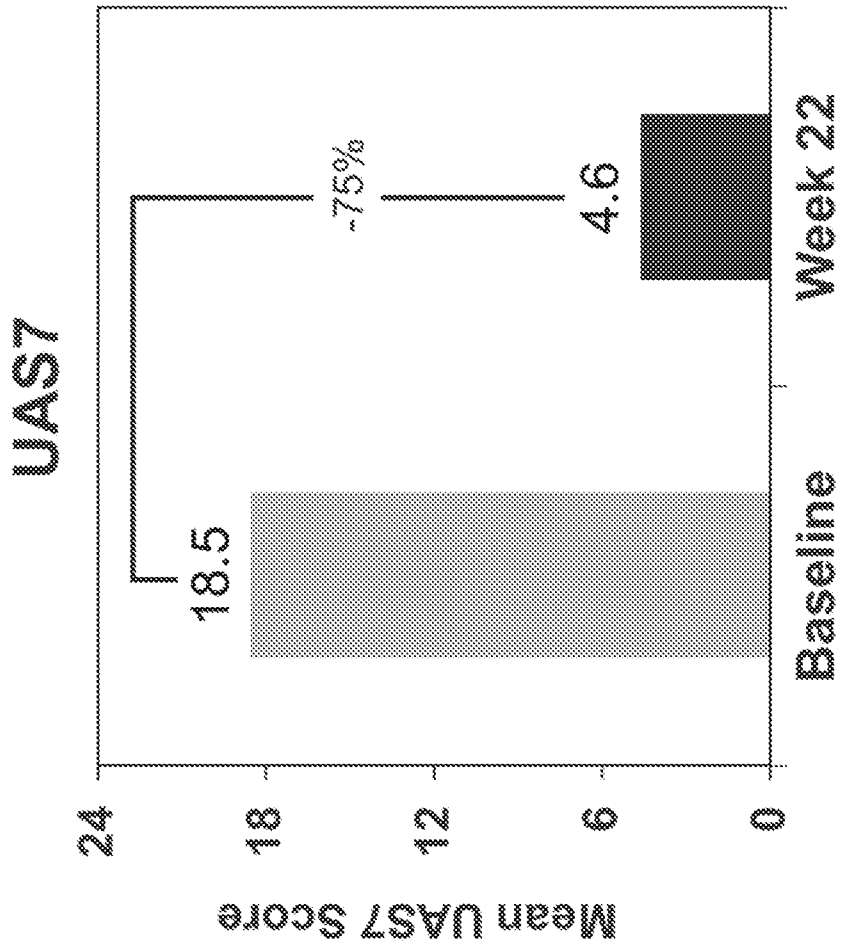


FIG. 2A

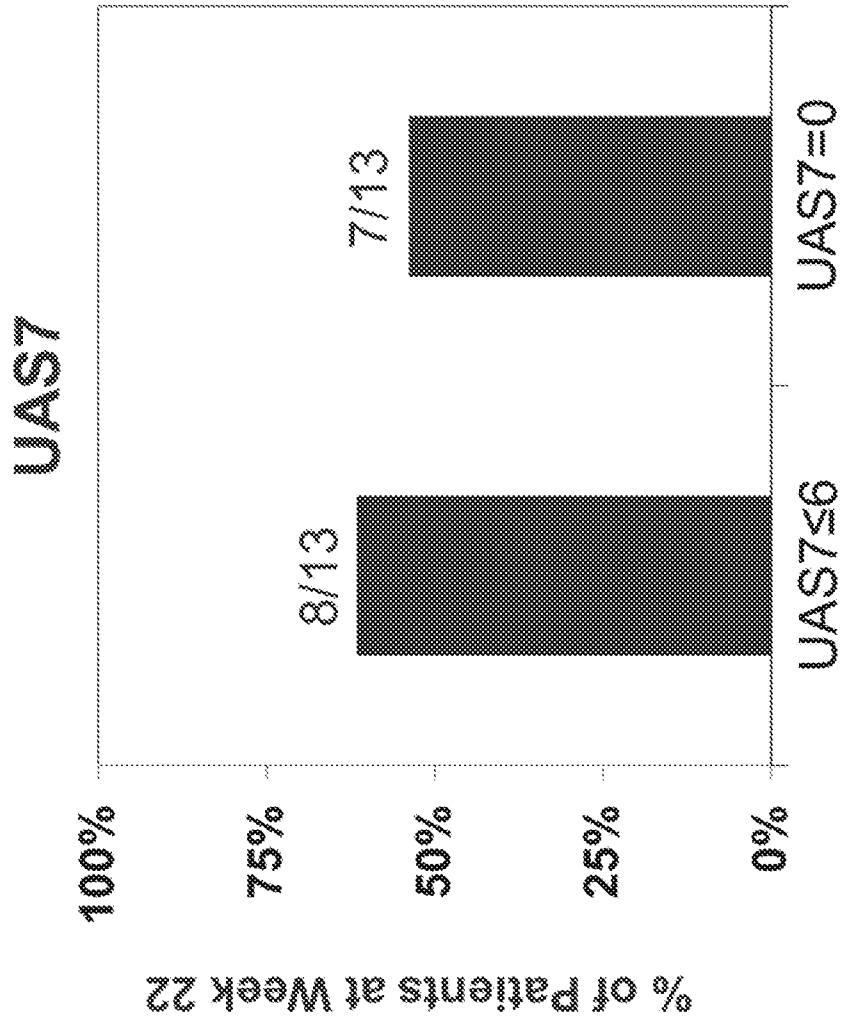


FIG. 2B

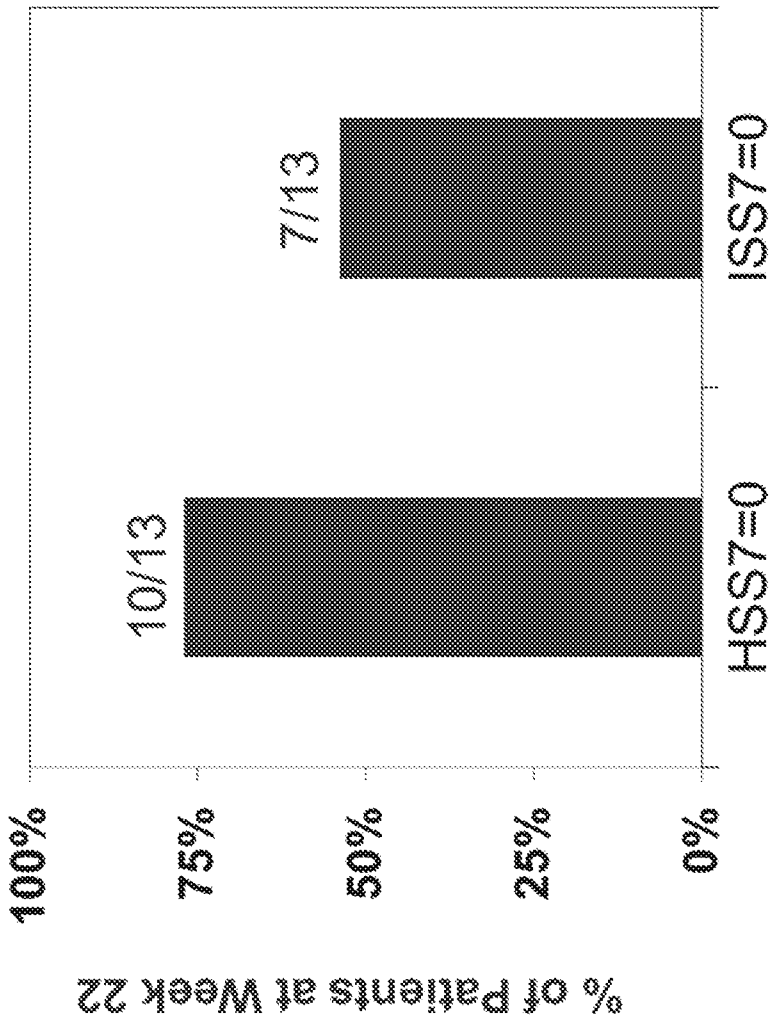
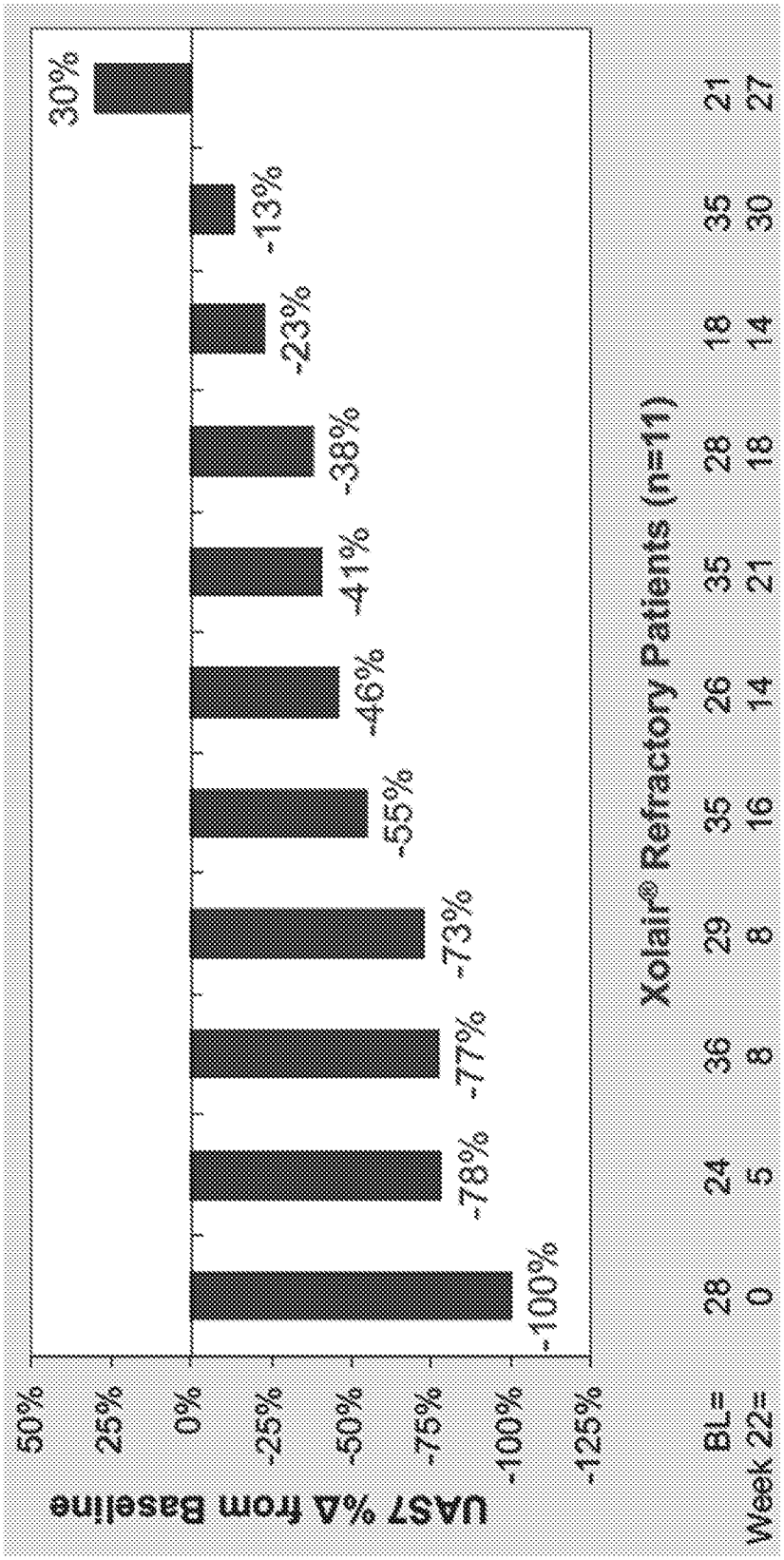


FIG. 2C


UAS7 %Δ from Baseline
50%
25%
0%
-25%
-50%
-75%
-100%
-125%
30%
-13%
-23%
-38%
-41%
-46%
-55%
-73%
-77%
-78%
-100%
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BL= 28 24 36 29 35 26 35 28 18 35 21
Week 22= 0 5 8 8 16 14 21 18 14 30 27

FIG. 3

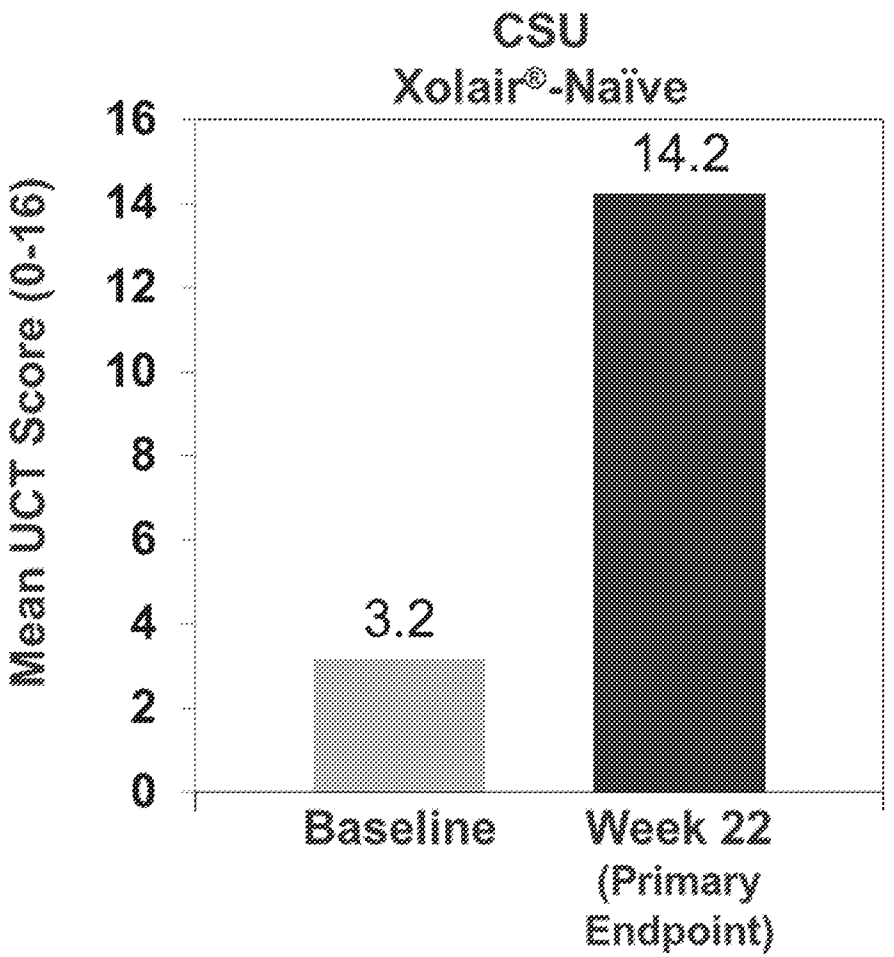
CSU
Xolair®-Naïve
Mean UCT Score (0-16)
16
14
12
10
8
6
4
2
0
14.2
3.2
Baseline
Week 22
(Primary
Endpoint)

FIG. 1B