(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 May 2010 (14.05.2010)

PCT

(10) International Publication Number
WO 2010/054403 A1

(51) International Patent Classification:
A61K 39/00 (2006.01) *C07K 16/24* (2006.01)
A61K 38/00 (2006.01)

(21) International Application Number:
PCT/US2009/063929

(22) International Filing Date:
10 November 2009 (10.11.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/198,803	10 November 2008 (10.11.2008)	US
61/199,563	18 November 2008 (18.11.2008)	US
61/199,569	18 November 2008 (18.11.2008)	US
61/199,562	18 November 2008 (18.11.2008)	US
61/199,764	19 November 2008 (19.11.2008)	US
61/200,634	1 December 2008 (01.12.2008)	US
61/200,635	1 December 2008 (01.12.2008)	US
61/200,640	1 December 2008 (01.12.2008)	US
61/181,788	28 May 2009 (28.05.2009)	US
61/228,047	23 July 2009 (23.07.2009)	US

(71) Applicant *(for all designated States except US)*: **ALEXION PHARMACEUTICALS, INC.** [US/US]; 352 Knotter Drive, Cheshire, CT 06410 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ROTHER, Russell, P.** [US/US]; 10325 Forest Meadow Drive, Oklahoma City, OK 73151 (US). **BEDROSIAN, Camille** [US/US]; 22 Lantern Road, Belmont, MA 02478 (US). **SQUINTO, Stephen, P.** [US/US]; 16 Coachmans Lane, Bethnay, CT 06524 (US). **BELL, Leonard** [US/US]; 59 Tumblebrook Road, Woodbridge, CT 06525 (US).

(74) Agents: **MURPHEY, Ryan** et al.; Ropes & Gray Llp, One International Place, Boston, MA 02110 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with amended claims (Art. 19(1))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2010/054403 A1

(54) **Title**: METHODS AND COMPOSITIONS FOR TREATING COMPLEMENT-ASSOCIATED DISORDERS

(57) **Abstract**: The present disclosure relates to, inter alia, compositions containing an inhibitor of human complement and use of the compositions in methods for treating or preventing complement-associated disorders. In some embodiments, the inhibitor is chronically administered to patients. In some embodiments, the inhibitor is administered to a patient in an amount and with a frequency to maintain systemic complement inhibition and prevent breakthrough. In some embodiments, the compositions contain an antibody, or antigen-binding fragment thereof, that binds to a human complement component C5 protein or a fragment of the protein such as C5a or C5b.

METHODS AND COMPOSITIONS FOR TREATING COMPLEMENT-ASSOCIATED DISORDERS

Cross-Reference to Related Applications

This application claims the benefit of U.S. Provisional Patent Application Serial Nos.: 61/198,803, filed on November 10, 2008; 61/199,563, filed on November 18, 2008; 61/199,562, filed on November 18, 2008; 61/199,569, filed on November 18, 2008; 61/199,764, filed on November 19, 2008; 61/200,640, filed on December 1, 2008; 61/200,634, filed on December 1, 2008; 61/200,635, filed on December 1, 2008; 61/181,788, filed on May 28, 2009; and 61/228,047, filed on July 23, 2009, the disclosures of each of which are hereby incorporated by reference in their entirety.

Technical Field

The field of the invention is medicine, immunology, molecular biology, and protein chemistry.

Background

The complement system acts in conjunction with other immunological systems of the body to defend against intrusion of cellular and viral pathogens. There are at least 25 complement proteins, which are found as a complex collection of plasma proteins and membrane cofactors. The plasma proteins make up about 10% of the globulins in vertebrate serum. Complement components achieve their immune defensive functions by interacting in a series of intricate but precise enzymatic cleavage and membrane binding events. The resulting complement cascade leads to the production of products with opsonic, immunoregulatory, and lytic functions. A concise summary of the biologic activities associated with complement activation is provided, for example, in The Merck Manual, 16th Edition.

The complement cascade can progress via the classical pathway (CP), the lectin pathway, or the alternative pathway (AP). The lectin pathway is typically initiated with binding of mannose-binding lectin (MBL) to high mannose substrates. The AP can be antibody independent, and can be initiated by certain molecules on pathogen surfaces. The CP is typically initiated by antibody recognition of, and binding to, an antigenic site on a target cell. These pathways converge at the C3

convertase – the point where complement component C3 is cleaved by an active protease to yield C3a and C3b.

The AP C3 convertase is initiated by the spontaneous hydrolysis of complement component C3, which is abundant in the plasma in the blood. This process, also known as "tickover," occurs through the spontaneous cleavage of a thioester bond in C3 to form C3i or C3(H_2O). Tickover is facilitated by the presence of surfaces that support the binding of activated C3 and/or have neutral or positive charge characteristics (e.g., bacterial cell surfaces). This formation of C3(H_2O) allows for the binding of plasma protein Factor B, which in turn allows Factor D to cleave Factor B into Ba and Bb. The Bb fragment remains bound to C3 to form a complex containing C3(H_2O)Bb – the "fluid-phase" or "initiation" C3 convertase. Although only produced in small amounts, the fluid-phase C3 convertase can cleave multiple C3 proteins into C3a and C3b and results in the generation of C3b and its subsequent covalent binding to a surface (e.g., a bacterial surface). Factor B bound to the surface-bound C3b is cleaved by Factor D to thus form the surface-bound AP C3 convertase complex containing C3b,Bb. (See, e.g., Müller-Eberhard (1988) *Ann Rev Biochem* 57:321-347.)

The AP C5 convertase – $(C3b)_2$,Bb – is formed upon addition of a second C3b monomer to the AP C3 convertase. (See, e.g., Medicus et al. (1976) *J Exp Med* 144:1076-1093 and Fearon et al. (1975) *J Exp Med* 142:856-863.) The role of the second C3b molecule is to bind C5 and present it for cleavage by Bb. (See, e.g., Isenman et al. (1980) *J Immunol* 124:326-331.) The AP C3 and C5 convertases are stabilized by the addition of the trimeric protein properdin as described in, e.g., Medicus et al. (1976), *supra*. However, properdin binding is not required to form a functioning alternative pathway C3 or C5 convertase. (See, e.g., Schreiber et al. (1978) *Proc Natl Acad Sci USA* 75: 3948-3952 and Sissons et al. (1980) *Proc Natl Acad Sci USA* 77: 559-562.)

The CP C3 convertase is formed upon interaction of complement component C1, which is a complex of C1q, C1r, and C1s, with an antibody that is bound to a target antigen (e.g., a microbial antigen). The binding of the C1q portion of C1 to the antibody-antigen complex causes a conformational change in C1 that activates Clr. Active C1r then cleaves the C1-associated C1s to thereby generate an active serine protease. Active C1s cleaves complement component C4 into C4b and C4a. Like

C3b, the newly generated C4b fragment contains a highly reactive thiol that readily forms amide or ester bonds with suitable molecules on a target surface (e.g., a microbial cell surface). C1s also cleaves complement component C2 into C2b and C2a. The complex formed by C4b and C2a is the CP C3 convertase, which is capable of processing C3 into C3a and C3b. The CP C5 convertase – C4b,C2a,C3b – is formed upon addition of a C3b monomer to the CP C3 convertase. (See, e.g., Müller-Eberhard (1988), *supra* and Cooper et al. (1970) *J Exp Med* 132:775-793.)

In addition to its role in C3 and C5 convertases, C3b also functions as an opsonin through its interaction with complement receptors present on the surfaces of antigen-presenting cells such as macrophages and dendritic cells. The opsonic function of C3b is generally considered to be one of the most important anti-infective functions of the complement system. Patients with genetic lesions that block C3b function are prone to infection by a broad variety of pathogenic organisms, while patients with lesions later in the complement cascade sequence, i.e., patients with lesions that block C5 functions, are found to be more prone only to *Neisseria* infection, and then only somewhat more prone.

The AP and CP C5 convertases cleave C5, which is a 190 kDa beta globulin found in normal serum at approximately 75 μg/ml (0.4 μM). C5 is glycosylated, with about 1.5-3 percent of its mass attributed to carbohydrate. Mature C5 is a heterodimer of a 999 amino acid 115 kDa alpha chain that is disulfide linked to a 655 amino acid 75 kDa beta chain. C5 is synthesized as a single chain precursor protein product of a single copy gene (Haviland et al. (1991) *J. Immunol.* 146:362-368). The cDNA sequence of the transcript of this gene predicts a secreted pro-C5 precursor of 1658 amino acids along with an 18 amino acid leader sequence (see, e.g., U.S. Patent No. 6,355,245).

The pro-C5 precursor is cleaved after amino acids 655 and 659, to yield the beta chain as an amino terminal fragment (amino acid residues +1 to 655 of the above sequence) and the alpha chain as a carboxyl terminal fragment (amino acid residues 660 to 1658 of the above sequence), with four amino acids (amino acid residues 656-659 of the above sequence) deleted between the two.

C5a is cleaved from the alpha chain of C5 by either alternative or classical C5 convertase as an amino terminal fragment comprising the first 74 amino acids of the alpha chain (i.e., amino acid residues 660-733 of the above sequence).

Approximately 20 percent of the 11 kDa mass of C5a is attributed to carbohydrate. The cleavage site for convertase action is at, or immediately adjacent to, amino acid residue 733 of the above sequence. A compound that would bind at, or adjacent, to this cleavage site would have the potential to block access of the C5 convertase enzymes to the cleavage site and thereby act as a complement inhibitor.

C5 can also be activated by means other than C5 convertase activity. Limited trypsin digestion (see, e.g., Minta and Man (1997) *J Immunol.* 119:1597-1602 and Wetsel and Kolb (1982) *J Immunol.* 128:2209-2216) and acid treatment (Yamamoto and Gewurz (1978) *J Immunol.* 120:2008 and Damerau et al. (1989) *Molec. Immunol.* 26:1133-1142) can also cleave C5 and produce active C5b.

Cleavage of C5 releases C5a, a potent anaphylatoxin and chemotactic factor, and leads to the formation of the lytic terminal complement complex, C5b-9. C5a and C5b-9 also have pleiotropic cell activating properties, by amplifying the release of downstream inflammatory factors, such as hydrolytic enzymes, reactive oxygen species, arachidonic acid metabolites and various cytokines.

The first step in the formation of the terminal complement complex involves the combination of C5b with C6, C7, and C8 to form the C5b-8 complex at the surface of the target cell. Upon the binding of the C5b-8 complex with several C9 molecules, the membrane attack complex (MAC, C5b-9, terminal complement complex--TCC) is formed. When sufficient numbers of MACs insert into target cell membranes the openings they create (MAC pores) mediate rapid osmotic lysis of the target cells. Lower, non-lytic concentrations of MACs can produce other effects. In particular, membrane insertion of small numbers of the C5b-9 complexes into endothelial cells and platelets can cause deleterious cell activation. In some cases activation may precede cell lysis.

As mentioned above, C3a and C5a are anaphylatoxins. These activated complement components can trigger mast cell degranulation, which releases histamine from basophils and mast cells, and other mediators of inflammation, resulting in smooth muscle contraction, increased vascular permeability, leukocyte activation, and other inflammatory phenomena including cellular proliferation resulting in hypercellularity. C5a also functions as a chemotactic peptide that serves to attract pro-inflammatory granulocytes to the site of complement activation.

C5a receptors are found on the surfaces of bronchial and alveolar epithelial cells and bronchial smooth muscle cells. C5a receptors have also been found on eosinophils, mast cells, monocytes, neutrophils, and activated lymphocytes.

While a properly functioning complement system provides a robust defense against infecting microbes, inappropriate regulation or activation of complement has been implicated in the pathogenesis of a variety of disorders including, e.g., rheumatoid arthritis (RA); lupus nephritis; ischemia-reperfusion injury; atypical hemolytic uremic syndrome (aHUS); dense deposit disease (DDD); macular degeneration (e.g., age-related macular degeneration (AMD)); hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome; thrombotic thrombocytopenic purpura (TTP); spontaneous fetal loss; Pauci-immune vasculitis; epidermolysis bullosa; recurrent fetal loss; multiple sclerosis (MS); traumatic brain injury; and injury resulting from myocardial infarction, cardiopulmonary bypass and hemodialysis. (See, e.g., Holers et al. (2008) *Immunological Reviews* 223:300-316.)

Summary

The present disclosure relates to compositions containing an inhibitor of human complement (e.g., an inhibitor of complement component C5 such as an anti-C5 antibody) and methods for using the compositions to treat or prevent complement-associated disorders. In some embodiments, the compositions contain an antibody, or antigen-binding fragment thereof, that binds to a human complement component C5 protein. In some embodiments, the compositions contain an antibody, or antigen-binding fragment thereof, that binds to human C5 fragment C5a or C5b. In some embodiments, the C5 inhibitor is a small molecule or a nucleic acid such as, e.g., a siRNA or an anti-sense RNA that binds to and promotes inactivation of C5 mRNA in a mammal.

Complement-associated disorders include any medical disorder in a human, the treatment of which would benefit directly or indirectly from inhibition of the complement system. The disorders are generally characterized by inappropriate regulation of the complement system such as inappropriate: (i) activation of the complement system or (ii) duration of an activated complement system in a subject. Complement-associated disorders include, without limitation, inflammatory and autoimmune disorders. A complement-associated disorder can be, e.g., RA;

antiphospholipid antibody syndrome (APS); lupus nephritis; ischemia-reperfusion injury; aHUS; typical (also referred to as diarrheal or infectious) hemolytic uremic syndrome (tHUS); DDD; neuromyelitis optica (NMO); multifocal motor neuropathy (MMN); MS; macular degeneration (e.g., AMD); HELLP syndrome; TTP; spontaneous fetal loss; Pauci-immune vasculitis; epidermolysis bullosa; recurrent fetal loss; and traumatic brain injury. In some embodiments, the complement-associated disorder is a complement-associated vascular disorder such as a cardiovascular disorder, myocarditis, a cerebrovascular disorder, a peripheral (e.g., musculoskeletal) vascular disorder, a renovascular disorder, a mesenteric/enteric vascular disorder, vasculitis, Henoch-Schönlein purpura nephritis, systemic lupus erythematosus-associated vasculitis, vasculitis associated with rheumatoid arthritis, immune complex vasculitis, Takayasu's disease, dilated cardiomyopathy, diabetic angiopathy, Kawasaki's disease (arteritis), venous gas embolus (VGE), and restenosis following stent placement, rotational atherectomy, and percutaneous transluminal coronary angioplasty (PTCA). Additional complement-associated disorders include, without limitation, myasthenia gravis (MG), cold agglutinin disease (CAD), dermatomyositis, paroxysmal cold hemoglobinuria (PCH), Graves' disease, atherosclerosis, Alzheimer's disease, systemic inflammatory response sepsis, septic shock, spinal cord injury, glomerulonephritis, Hashimoto's thyroiditis, type I diabetes, psoriasis, pemphigus, autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), Goodpasture syndrome, Degos disease, and catastrophic APS (CAPS).

In one aspect, the disclosure features a method for treating or preventing a complement-associated disorder in a human. The method includes administering to a human in need thereof a therapeutically effective amount of a composition comprising an inhibitor of human complement (e.g., an inhibitor of human complement component C5).

In another aspect, the disclosure features a method for treating or preventing a complement-associated disorder in a human, which method comprises administering to a human in need thereof a composition comprising a therapeutically effective amount of an inhibitor of human complement (e.g., an inhibitor of human complement component C5).

In some embodiments of any of the methods described herein, the inhibitor can inhibit the expression of a human complement component C5 protein. The

inhibitor can inhibit the protein expression of a human complement component C5 protein or inhibit the expression of an mRNA encoding the protein. In some embodiments of any of the methods described herein, the inhibitor can inhibit the cleavage of human complement component C5 into fragments C5a and C5b.

In some embodiments of any of the methods described herein, the inhibitor binds to, and inhibits, one or both of C5a and C5b. The inhibitor can be, e.g., an antibody that binds to C5a or C5b. In some embodiments, the inhibitor is an antibody that binds to C5a, but does not bind to full-length C5. In some embodiments, the inhibitor is an antibody that binds to C5b, but does not bind to full-length C5. In some embodiments, the inhibitor is an antibody that binds to a human C5a protein or a fragment thereof having an amino acid sequence that contains, or consists of, at least four (e.g., at least four, five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 16, or 17 or more) consecutive amino acids depicted in any one of SEQ ID NOs:12-25. In some embodiments, the inhibitor is an antibody that binds to human C5a protein having the amino acid sequence depicted in SEQ ID NO:12. In some embodiments, the inhibitor is an antibody that binds to a human C5b protein or fragment thereof having an amino acid sequence that contains, or consists of, at least four (e.g., at least four, five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 16, or 17 or more) consecutive amino acids depicted in any one of SEQ ID NOs:4 or 26. In some embodiments, the inhibitor is an antibody that binds to human C5b protein having the amino acid sequence depicted in SEQ ID NO:4 or 26.

In some embodiments of any of the methods described herein, the inhibitor can be selected from the group consisting of a polypeptide, a polypeptide analog, a nucleic acid, a nucleic acid analog, and a small molecule. The polypeptide can be, or consist of, an antibody, or antigen-binding fragment thereof, that binds to a human complement component C5 protein such as any of those described herein. In some embodiments, the antibody can bind to the alpha chain of the complement component C5 protein. In some embodiments, the antibody can bind to the beta chain of the complement component C5 protein. In some embodiments, the antibody can bind to the alpha chain of human complement component C5, and the antibody can (i) inhibit complement activation in a human body fluid, (ii) inhibit the binding of purified human complement component C5 to either human complement component C3b or human complement component C4b, and/or (iii) not bind to the human complement

activation product free C5a (or a combination of any of the foregoing properties). The antibody can bind to the human complement component C5 protein having, or consisting of, the amino acid sequence depicted in any one of SEQ ID NOs:1-11. The antibody can bind to an isolated oligopeptide comprising an amino acid sequence corresponding to amino acid position 8 through amino acid position 12 of SEQ ID NO:5. In some embodiments, the antibody can be a monoclonal antibody, a single-chain antibody, a humanized antibody, a fully human antibody, a polyclonal antibody, a recombinant antibody, a diabody, a chimerized or chimeric antibody, a deimmunized human antibody, a fully human antibody, a single chain antibody, an Fv fragment, an Fd fragment, an Fab fragment, an Fab' fragment, or an $F(ab')_2$ fragment. In some embodiments, the antibody can be eculizumab or pexelizumab.

In some embodiments of any of the methods described herein, the composition can be intravenously administered to the human.

In some embodiments of any of the methods described herein, the complement-associated disorder is an alternative complement pathway-associated disorder. In some embodiments of any of the methods described herein, the complement-associated disorder is a classical complement pathway-associated disorder. In some embodiments, the complement-associated disorder is selected from the group consisting of rheumatoid arthritis, ischemia-reperfusion injury, atypical hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, dense deposit disease, age-related macular degeneration, spontaneous fetal loss, Pauci-immune vasculitis, epidermolysis bullosa, recurrent fetal loss, multiple sclerosis, HELLP, pre-eclampsia, traumatic brain injury, Alzheimer's disease, myasthenia gravis, cold agglutinin disease, dermatomyositis, Graves' disease, Hashimoto's thyroiditis, type I diabetes, psoriasis, pemphigus, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, Goodpasture syndrome, antiphospholipid syndrome, catastrophic antiphospholipid syndrome, neuromyelitis optica (NMO), multifocal motor neuropathy (MMN), Degos disease, and any other complement-associated disorder described herein.

In some embodiments, any of the methods described herein can further include the step of identifying the human as having, suspected of having, or at risk for developing, a complement-associated disorder. In some embodiments, any of the methods described herein can also include, after the administering, monitoring the

human for an improvement in one or more symptoms of the complement-associated disorder.

In embodiments of any of the methods described herein where the complement-associated disorder is aHUS, the aHUS can be genetic, acquired, or idiopathic form. In some embodiments, the aHUS can be complement factor H (CFH)-associated aHUS (e.g., due to mutations in CFH or the presence of antibodies in the subject that bind to CFH), membrane cofactor protein (MCP)-associated aHUS, complement factor I (CFI)-associated aHUS, C4b-binding protein (C4BP)-associated aHUS, a von Willibrand Factor (vWF)-associated disorder, complement factor B-(CFB)-associated aHUS, or a disorder of the alternative pathway that results in low C3 levels as a result of increased C3 consumption.

In some embodiments, any of the methods described herein can further include identifying the subject as one having, suspected of having, or at risk for developing, aHUS.

In some embodiments, any of the methods described herein can include, after the administering, monitoring the subject for an improvement in one or more symptoms of aHUS.

In some embodiments of any of the methods described herein, the composition can be administered to the subject prior to, during, or following a plasma therapy (e.g., plasma exchange or plasma infusion). In some embodiments, administration of the C5 inhibitor to the subject can alleviate the need for plasma therapy by a patient. For example, in some embodiments, administration (e.g., chronic administration) of the C5 inhibitor to the subject can alleviate or substantially reduce the need for plasma therapy by a patient for at least 2 months (e.g., 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months or 1, 2, 3, 4, 5, or 6 years or more). In some embodiments, any of the methods described herein can include administering to the subject one or more additional active agents useful for treating typical HUS or aHUS. The one or more additional active agents can be, e.g., selected from the group consisting of anti-hypertensives, anti-platelet agents, prostacyclin, fibrinolytic agents, and anti-oxidants.

In some embodiments, the human is an infant. The infant can be, e.g., 0.5 (e.g., 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, or 9.5) years old. The

infant can be less than 10 (e.g., less than 9.5, 9, 8.5, 8, 7.5, 7, 6.5, 6, 5.5, 5, 4.5, 4, 3.5, 3, 2.5, 2, 1.5, 1, or less than 1) year(s) old.

In embodiments of any of the methods described herein where the complement-associated disorder is typical HUS, the typical HUS can be associated with an *E. coli* infection in or on the human. The *E. coli* infection can be, e.g., an *E. coli* O157 (e.g., O157:H7), O26, O103, O111, or O145 infection. In some embodiments of any of the methods described herein, the typical hemolytic uremic syndrome can be associated with a *Shigella dysenteriae* infection in or on the human. The *Shigella dysenteriae* infection can be a *Shigella dysenteriae* type 1 infection.

In some embodiments, any of the methods described herein can further include identifying the human as one having, suspected of having, or at risk for developing, typical hemolytic uremic syndrome.

In some embodiments, any of the methods described herein can include, after the administering, monitoring the human for an improvement in one or more symptoms of typical hemolytic uremic syndrome.

In embodiments of any of the methods described herein where the complement-associated disorder is CAPS, the CAPS can be associated with a precipitating condition. Precipitating conditions can include, e.g., a cancer, transplantation, an infection, surgery, primary antiphospholipid syndrome, or an autoimmune disorder such as rheumatoid arthritis or systemic lupus erythematosus. Accordingly, in some embodiments, the CAPS can be associated with a cancer such as, but not limited to, gastric cancer, ovarian cancer, lymphoma, leukemia, endometrial cancer, adenocarcinoma, lung cancer, or any other cancers known in the art to precipitate or be associated with CAPS. In some embodiments, the CAPS can be idiopathic.

In some embodiments, any of the methods described herein can also include identifying the human as one having, suspected of having, or at risk for developing, CAPS. In some embodiments, any of the methods described herein can include, after the administering, monitoring the human for an improvement in one or more symptoms of CAPS.

In some embodiments of any of the methods described herein, the composition can be administered to the human prior to, during, or following a plasma exchange, plasmapheresis, IVIG, or any other additional therapy for treating CAPS.

In some embodiments, any of the methods described herein can also include administering to the human one or more additional active agents useful for treating CAPS. The one or more additional active agents can be selected from the group consisting of anti-hypertensives, anti-cytokine agents, steroids, anti-coagulants, or fibrinolytic agents.

In embodiments of any of the methods described herein where the complement-associated disorder is TTP, the TTP can be inherited. For example, a human can carry one or more (e.g., two, three, four, or five or more) mutations in the ADAMTS13 gene. In some embodiments of any of the methods described herein, the TTP can be an acquired form. For example, in some embodiments, the human can produce antibodies that bind to, and inhibit, the ADAMTS13 metalloproteinase. In some embodiments of any of the methods described herein, the TTP can be a recurrent form. For example, the human can be one who has had TTP. In some embodiments of any of the methods described herein, the TTP (or recurrent TTP) is associated with a precipitating condition such as, but not limited to, a cancer, pregnancy, bacterial or viral infection, surgery, or any other TTP-associated condition known in the art or described herein. In some embodiments of any of the methods described herein, the TTP (or recurrent TTP) is associated with the use of a therapeutic agent associated with TTP. For example, the TTP can be associated with the use of, e.g., a platelet aggregation inhibitor such as ticlopidine or clopidogrel or an immunosuppressant (e.g., cyclosporine, mitomycin C, FK506, or interferon-alpha).

In some embodiments, any of the methods described herein can include identifying the human as one having, suspected of having, or at risk for developing, TTP. In some embodiments, any of the methods described herein can include, after the administering, monitoring the human for an improvement in one or more symptoms of TTP.

In some embodiments of any of the methods described herein, the composition can be administered to the human prior to, during, or following a plasma exchange, plasma infusion, plasmapheresis, or a splenectomy. In some embodiments, any of the methods described herein can include administering to the human one or more additional active agents useful for treating or preventing TTP. The one or more additional active agents can be selected from the group consisting of anti-hypertensives, steroids, anti-coagulants, or fibrinolytic agents.

In embodiments of any of the methods described herein where the complement-associated disorder is DDD, the DDD can be an inherited form of the disorder. For example, a human can have a DDD-associated mutation in the complement factor H gene, the complement factor H-related 5 gene, or the complement component C3 gene.

In some embodiments, any of the methods described herein can include identifying the human as one having, suspected of having, or at risk for developing, DDD. In some embodiments, any of the methods described herein can include, after the administering, monitoring the human for an improvement in one or more symptoms of DDD.

In some embodiments of any of the methods described herein, the composition can be administered to the human prior to, during, or following a plasma exchange, plasma replacement, plasmapheresis, or intravenous gamma globulin therapy. In some embodiments, any of the methods described herein can include administering to the human one or more additional active agents useful for treating DDD. The one or more additional active agents can be selected from the group consisting of anti-hypertensives, corticosteroids, anti-coagulants, or fibrinolytic agents.

In embodiments of any of the methods described herein where the complement-associated disorder is MG, the human can be one expressing an MG-associated autoantibody such as, but not limited to, an MG-associated anti-AChR antibody, an MG-associated anti-MuSK antibody, or an MG-associated anti-striational protein antibody. The MG can be ocular MG and/or a drug-induced form of MG such as D-penicillamine-induced MG. In some embodiments, the human can be in, or be at risk for developing, myasthenic crisis. In some embodiments, the human can be a neonate having neonatal MG, wherein a mother with MG passes MG-associated antibodies through the placenta to an infant.

In some embodiments, any of the methods described herein can further include identifying the human as one having, suspected of having, or at risk for developing, MG. In some embodiments, any of the methods described herein can further include, after the administering, monitoring the human for an improvement in one or more symptoms of MG. In some embodiments of any of the methods described herein, the composition can be administered to the human prior to, during, or following a plasma exchange, plasmapheresis, IVIG, or immunoadsorption therapy.

In some embodiments, any of the methods described herein can include administering to the human one or more additional active agents useful for treating or preventing MG. The one or more additional active agents can be, e.g., acetylcholinesterase inhibitors, immunosuppressive agents, or any other additional active agents useful for treating MG that are known in the art or described herein.

In embodiments of any of the methods described herein where the complement-associated disorder is paroxysmal cold hemoglobinuria (PCH), the PCH can be associated with an infection (e.g., a viral or bacterial infection) or a neoplasm. For example, the PCH can be associated with a *Treponema palladium* infection, an influenza virus infection, a varicella-zoster virus infection, a cytomegalovirus (CMV) infection, an Epstein-Barr virus (EBV) infection, an adenovirus infection, a parvovirus B19 infection, a Coxsackie A9 infection, a *Haemophilus influenza* infection, a *Mycoplasma pneumoniae* infection, or a *Klebsiella pneumoniae* infection. In some embodiments, the PCH can be associated with non-Hodgkin's lymphoma. In some embodiments, the PCH can be associated with an immunization (e.g., a measles immunization). In some embodiments of any of the methods described herein, the PCH can be acute or recurrent.

In some embodiments, any of the methods described herein can include identifying the human as one having, suspected of having, or at risk for developing, PCH. In some embodiments, any of the methods described herein can include, after the administering, monitoring the human for an improvement in one or more symptoms of PCH.

In some embodiments of any of the methods described herein, the composition can be administered to the human prior to, during, or following a plasma exchange, plasma infusion, IVIG therapy, red cell transfusion, or plasmapheresis. In some embodiments, any of the methods described herein can include administering to the human one or more additional active agents useful for treating or preventing PCH. The one or more additional active agents can be selected from the group consisting of anti-hypertensives, steroids, immunosuppressives (e.g., rituximab), antibiotics, anti-viral agents, and chemotherapeutic agents.

In embodiments of any of the methods described herein where the complement-associated disorder is CAD, the CAD can be associated with an infection (e.g., a viral or bacterial infection) or a neoplasm. For example, the CAD can be

associated with an HIV infection, a cytomegalovirus (CMV) infection, an Epstein-Barr virus (EBV) infection, or a *Mycoplasma pneumoniae* infection. In some embodiments, the CAD can be associated with non-Hodgkin's lymphoma. In some embodiments of any of the methods described herein, the CAD can be primary or secondary.

In some embodiments, any of the methods described herein can include identifying the human as one having, suspected of having, or at risk for developing, CAD. In some embodiments, any of the methods described herein can include, after the administering, monitoring the human for an improvement in one or more symptoms of CAD.

In some embodiments of any of the methods described herein, the composition can be administered to the human prior to, during, or following a plasma exchange, plasma replacement, IVIG therapy, or plasmapheresis. In some embodiments, any of the methods described herein can include administering to the human one or more additional active agents useful for treating or preventing CAD. The one or more additional active agents can be selected from the group consisting of anti-hypertensives, steroids, immunosuppressives (e.g., rituximab), antibiotics, anti-viral agents, and chemotherapeutic agents.

In embodiments of any of the methods described herein where the complement-associated disorder is HELLP syndrome, the affected woman can be pregnant or can be a woman who has recently been pregnant. For example, the woman can be one who has given birth less than 14 days (e.g., less than 13 days, 12 days, 11 days, 10 days, nine days, eight days, seven days, six days, five days, four days, three days, two days, 24 hours, 18 hours, 12 hours, 6 hours, or less than 4, 3, 2, or 1 hours) prior to administration. In some embodiments, the woman has been pregnant more than one time. In some embodiments, the woman can be one who has developed preeclampsia or HELLP syndrome during at least one prior pregnancy.

In embodiments where the complement-associated disorder is HELLP syndrome, the methods described herein can further include the step of identifying the woman as one having, suspected of having, or at risk for developing, HELLP syndrome. In some embodiments, any of the methods described herein can further include the step of, after the administering, monitoring the woman for an improvement in one or more symptoms of HELLP syndrome.

In some embodiments of any of the methods described herein, the composition can be administered to the woman prior to, during, or following a plasma exchange, plasmapheresis, platelet transfusion, or red blood cell transfusion.

In some embodiments, any of the methods described herein can include the step of administering to the woman at least one or more additional active agents useful for treating or preventing HELLP syndrome in a woman. The one or more additional active agents can be selected from the group consisting of an anti-hypertensive, a steroid, an anti-seizure agent, and an anti-thrombotic agent.

In yet another aspect, the disclosure features an article of manufacture, which includes (or consists of) a container with a label and a composition containing an inhibitor of human complement (e.g., an inhibitor of human complement component C5). The label indicates that the composition is to be administered to a human having, suspected of having, or at risk for developing, a complement-associated disorder such as any of the complement-associated disorders described herein. The inhibitor can be, e.g., an antibody or antigen-binding fragment thereof that binds to complement component C5 or a fragment thereof such as C5a or C5b. In some embodiments, the article of manufacture contains one or more additional active agents that are useful for treating or preventing a complement-associated disorder (e.g., ameliorating one or more symptoms of the disorder).

The disclosure is also based, in part, on the discovery by the inventors that upon treatment with the C5 inhibitor eculizumab, a patient with the complement-associated disorder aHUS and thrombotic microangiopathy (TMA) in the kidney experienced a complete resolution of the TMA in the kidney with no further development of TMA. Accordingly, in another aspect, the disclosure features a method for treating thrombotic microangiopathy (TMA), or reducing the occurrence or severity of TMA, in a patient who has, is suspected of having, or at risk of developing TMA. The method includes administering to the patient (being in need thereof) an inhibitor of complement such as an inhibitor of complement component C5 to thereby treat TMA in the patient. The inhibitor can be, e.g., any of the C5 inhibitors described herein, e.g., eculizumab. Administration of the C5 inhibitor can reduce the occurrence or severity of TMA in the brain and/or kidney of the patient. In some embodiments, administration of the C5 inhibitor treats or promotes the

resolution of pre-existing TMA in the patient, e.g., a pre-existing TMA in the brain or kidney of the patient.

In some embodiments, the patient has a complement associated-disorder such as any of those described herein, e.g., membranoproliferative glomerulonephritis, Degos disease, atypical hemolytic uremic syndrome, antibody-mediated rejection, HELLP syndrome, or catastrophic antiphospholipid syndrome.

The inventors have also discovered that administration of eculizumab to patients with, e.g., aHUS or CAPS results in an unexpectedly rapid amelioration of one or more symptoms of the diseases. For example, the inventors have discovered that hypertension, reduced urine output, and low platelet levels are ameliorated in eculizumab-treated aHUS patients in less than one month (e.g., less than two weeks) from initiating chronic treatment with eculizumab. In another example, the inventors discovered that the proteinuria in a patient with membranoproliferative glomerulonephritis was ameliorated within a month following initiation of chronic treatment with eculizumab. Accordingly, in yet another aspect, the disclosure features a method for ameliorating one or more symptoms associated with a complement-associated disorder such as any of the complement-associated disorders described herein with the exception of paroxysmal nocturnal hemoglobinuria. The method includes administering to a patient in need thereof an inhibitor of complement (e.g., an inhibitor of complement component C5) in an amount effective to ameliorate one or more symptoms associated a complement-associated disorder, wherein the symptoms are ameliorated within less than two months (e.g., less than 7, 6, 5, 4, 3, or 2 weeks; less than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 day(s); or less than 12, 11, 10, 9, 8, 7, 6 or even less than 5 hours) after administering the inhibitor. Symptoms of complement-associated disorders are well known in the art of medicine and described herein. The complement inhibitor can be any of the C5 inhibitors described herein, e.g., eculizumab. Exemplary symptoms that may be ameliorated by the C5 inhibitor in less than 2 months include, e.g., proteinuria, hypertension, reduced platelet counts, and reduced urine output from the kidney. In some embodiments, at least one of the symptoms is ameliorated to within 40 (e.g., 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or even 1) % of its normal level or value. For example, in some embodiments, administration of the C5 inhibitor eculizumab to

a hypertensive patient with aHUS can ameliorate the patient's hypertension to within 40% of the normal blood pressure (diastolic and/or systolic) for the patient. In some embodiments, administration of the C5 inhibitor can completely ameliorate one or more symptoms of the complement-associated disorder in the subject. In some embodiments, the patient has had a kidney transplant, e.g., an aHUS patient who has recently undergone a kidney transplant. The complement associated-disorder can be any of those described herein, e.g., membranoproliferative glomerulonephritis, Degos disease, atypical hemolytic uremic syndrome, antibody-mediated rejection, HELLP syndrome, and catastrophic antiphospholipid syndrome.

Many of the complement-associated disorders described herein are characterized by episodic or sporadic symptom presentation and historically have only been treated when symptoms manifest. However, the inventors have discovered that an underlying complement-associated disorder remains present even when the patients are asymptomatic. The inventors have also discovered that recurrences or relapses of the disorders can be prevented or at least minimized by chronic treatment using a complement-mediated inhibitor. Such chronic administration of the inhibitor is useful to prevent or minimize the often irreversible damage (e.g., loss of an organ such as a kidney) inflicted upon patients with severe complement-related disorders (e.g., aHUS or CAPS) when the relapses occur. Accordingly, it is of the utmost importance to administer a complement inhibitor to the patient in an amount and with a frequency sufficient to continually maintain a concentration of the inhibitor that is high enough to prevent or substantially inhibit systemic complement activity in the patients.

Thus, in another aspect, the disclosure features a method for treating a complement-associated disorder, which method includes chronically administering to a patient in need thereof a complement inhibitor (e.g., a C5 inhibitor such as an anti-C5 antibody) in an amount and with a frequency that are effective to maintain systemic complement inhibition in the patients with the proviso that the complement-associated disorder is not paroxysmal nocturnal hemoglobinuria.

As used herein, "chronically administered," "chronic treatment," "treating chronically," or similar grammatical variations thereof refer to a treatment regimen that is employed to maintain a certain threshold concentration of a therapeutic agent in the blood of a patient in order to completely or substantially suppress systemic

complement activity in the patient over a prolonged period of time. Accordingly, a patient chronically treated with a complement inhibitor can be treated for a period of time that is greater than or equal to 2 weeks (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 weeks; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, or 12 years or for the remainder of the patient's life) with the inhibitor in an amount and with a dosing frequency that are sufficient to maintain a concentration of the inhibitor in the patient's blood that inhibits or substantially inhibits systemic complement activity in the patient. In some embodiments, the complement inhibitor can be chronically administered to a patient in need thereof in an amount and with a frequency that are effective to maintain serum hemolytic activity at less than or equal to 20 (e.g., 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, or even below 5) % and to maintain serum hemolytic activity at less than or equal to 20%. *See*, e.g., Hill et al. (2005) *Blood* 106(7):2559. In some embodiments, the complement inhibitor can be administered to a patient in an amount and with a frequency that are effective to maintain serum lactate dehydrogenase (LDH) levels at within at least 20 (e.g., 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, or even below 5) % of the normal range for LDH. *See* Hill et al. (2005) *supra*. In some embodiments, the complement inhibitor is administered to the patient in an amount and with a frequency that are effective to maintain a serum LDH level less than 550 (e.g., less than 540, 530, 520, 510, 500, 490, 480, 470, 460, 450, 430, 420, 410, 400, 390, 380, 370, 360, 350, 340, 330, 320, 310, 300, 290, 280, or less than 270) IU/L. To maintain systemic complement inhibition in a patient, the complement inhibitor can be chronically administered to the patient, e.g., once a week, once every two weeks, twice a week, once a day, once a month, or once every three weeks. In some embodiments of any of the methods described herein, a C5 inhibitor (e.g., an anti-C5 antibody) can be administered to a patient in an amount and with a frequency of administration effective to maintain a concentration of at least 0.7 (e.g., at least 0.8, 0.9, one, two, three, four, five, six, seven, eight, nine, or 10 or more) divalent C5 inhibitor molecule(s) (e.g., a whole anti-C5 antibody such as eculizumab) per every C5 molecule in the patient's blood. "Divalent" or "bivalent," with respect to a C5 inhibitor, refers to a C5 inhibitor that contains at least two binding sites for a C5 molecule. Where the C5 inhibitor is

monovalent (e.g., a single chain anti-C5 antibody or a Fab that binds to C5), the inhibitor can be administered to the patient in an amount and with a frequency that are effective to maintain a concentration of at least 1.5 (e.g., at least 2, 2.5, 3, 3.5, 4, 4.5, or 5 or more) of the monovalent C5 inhibitors per every C5 molecule in the blood. In some embodiments, the monovalent C5 inhibitor can be administered to the patient in an amount and with a frequency that are effective to maintain a ratio of monovalent C5 inhibitor to C5 of at least 2:1 (e.g., at least 3:1, at least 4:1, at least 5:1, or at least 6:1 or more). In some embodiments of any of the methods described herein, a whole (bivalent) anti-C5 antibody is administered to the patient in an amount and with a frequency that are effective to maintain a concentration of at least 40 (e.g., 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 80, 85, 90, 95, 100, 110, or 120 or more) μg of the antibody per milliliter of the patient's blood. In preferred embodiments, a whole anti-C5 antibody (e.g., eculizumab) is administered in an amount and with a frequency to maintain the antibody at a concentration of at least 50 μg per milliliter of the patient's blood. In preferred embodiments, a whole anti-C5 antibody (e.g., eculizumab) is administered in an amount and with a frequency to maintain the antibody at a concentration of at least 100 μg per milliliter of the patient's blood. In some embodiments of any of the methods described herein, a monovalent anti-C5 antibody (e.g., a single chain antibody or an Fab fragment) can be administered to the patient in an amount and with a frequency that are effective to maintain a concentration of at least 80 (e.g., 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, or a 170 or more) μg of the antibody per milliliter of the patient's blood. Exemplary chronic dosing strategies are described herein.

In another aspect, the disclosure features a method for treating a complement-associated disorder, which method includes chronically administering to a patient in need thereof an anti-C5 antibody in an amount and with a frequency that are effective to maintain systemic complement inhibition in the patients. In some embodiments, the anti-C5 antibody can be chronically administered to a patient in need thereof in an amount and with a frequency that are effective to maintain serum hemolytic activity at less than or equal to 20 (e.g., 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, or even below 5) % and to maintain the serum hemolytic activity at less than or equal to 20%.

See, e.g., Hill et al. (2005) *Blood* 106(7):2559. In some embodiments, the anti-C5 antibody can be administered to a patient in an amount and with a frequency that are effective to maintain serum lactate dehydrogenase (LDH) levels at within: at least 20 (e.g., 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, or even below 5) % of the normal range for LDH; or less than or equal to 550 (e.g., less than or equal to 550, 540, 530, 520, 510, 500, 490, 480, 470, 460, 450, 430, 420, 410, 400, 390, 380, 370, 360, 350, 340, 330, 320, 310, 300, 290, 280, or less than 270) IU/L. *See*, e.g., Hill et al. (2005) *supra*. In some embodiments, the anti-C5 antibody is administered to the patient in an amount and with a frequency to maintain a concentration of at least 0.7 (e.g., at least 0.8, 0.9, 1, 2, 3, or 4 or more) whole (bivalent) anti-C5 antibody molecule(s) per every C5 molecule in the patient's blood. In some embodiments, the anti-C5 antibody can be administered to the patient in an amount and with a frequency that are effective to maintain a ratio of whole (bivalent) anti-C5 antibody to C5 in the blood of at least 1:1 (e.g., at least 3:2, 2:1, 5:2, or 3:1). Where the anti-C5 antibody is monovalent, the anti-C5 antibody can be administered to the patient in an amount and with a frequency that are effective to maintain a concentration of at least 2 of the monovalent anti-C5 antibodies per every C5 molecule in the blood. In some embodiments, the monovalent anti-C5 antibody can be administered to the patient in an amount and with a frequency that are effective to maintain a ratio of monovalent anti-C5 antibody to C5 of at least 2:1 (e.g., at least 3:1, at least 4:1, at least 5:1, or 6:1 or more). The anti-C5 antibody can be, e.g., eculizumab. The patient can have, be suspected of having, or be at risk for developing a complement-associated disorder with the proviso that the disorder is not paroxysmal nocturnal hemoglobinuria. For example, the complement-associated disorder can be one selected from the group consisting of membranoproliferative glomerulonephritis, Degos disease, atypical hemolytic uremic syndrome, antibody-mediated rejection, HELLP syndrome, and catastrophic antiphospholipid syndrome.

In some embodiments of any of the methods described herein, an anti-C5 antibody can be administered chronically to a patient based on his or her weight. In some embodiments of any of the methods described herein, an anti-C5 antibody (e.g., eculizumab) can be administered chronically to a patient based on his or her weight and under the dosing schedule set forth in Table 1.

Table 1. Exemplary Chronic Dosing Schedules for a Whole Anti-C5 Antibody (e.g., eculizumab) by Patient Weight

		Maintenance Dosing	
Patient Weight	**Induction/Loading Dosing**	**(A)**	**(B)**
Adults of any weight or any patient with a body weight that is greater than or equal to 40 kg	At least 800 (e.g., at least 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg once a week for four weeks	At least 800 (e.g., at least 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, or 1400 or more) mg on week five	Following the (A) dose, at least 800 (e.g., at least 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, or 1400 or more) mg once every two weeks thereafter*
Body weight that is less than 40 kg, but greater than or equal to 30 kg	At least 500 (e.g., at least 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, or 850 or more) mg once a week for two weeks	At least 800 (e.g., at least 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, or 1400 or more) mg on week three	Following the (A) dose, at least 800 (e.g., at least 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, or 1400 or more) mg once every two weeks thereafter*
Body weight that is less than 30 kg, but greater than or equal to 20 kg	At least 500 (e.g., at least 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, or 850 or more) mg once a week for two weeks	At least 500 (e.g., at least 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800,	Following the (A) dose, at least 500 (e.g., at least 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, or 850 or more) mg once every two

		Maintenance Dosing	
Patient Weight	**Induction/Loading Dosing**	**(A)**	**(B)**
		or 850 or more) mg on week three	weeks thereafter*
Body weight that is less than 20 kg, but greater than or equal to 10 kg	At least 500 (e.g., at least 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, or 850 or more) mg once a week for one week	At least 200 (e.g., at least 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, or 550 or more) mg on week two	Following the (A) dose, at least 200 (e.g., at least 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, or 550 or more) mg once every two weeks thereafter*
Body weight that is less than 10 kg, but greater than or equal to 5 kg	At least 200 (e.g., at least 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, or 550 or more) mg once a week for one week	At least 200 (e.g., at least 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, or 550 or more) mg on week two	Following the (A) dose, at least 200 (e.g., at least 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, or 550 or more) mg once every three weeks thereafter*

*In accordance with the present disclosure, the (B) maintenance dosing schedule can be maintained for the duration of the treatment regimen, e.g., at least one (e.g., at least two, three, four, five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 36, or 48 or more) month(s); at least one (e.g., at least two, three, four, five, six, seven, eight, nine, 10, 11, 12, 13, 14, or 15 or more) years; or for the remainder of the patient's life.

In preferred embodiments, an anti-C5 antibody (e.g., eculizumab) can be administered to a patient based on his or her weight under the dosing schedules set forth in Table 2.

Table 2. Exemplary Chronic Dosing Schedules for a Whole Anti-C5 Antibody (e.g., eculizumab) by Patient Weight

		Maintenance Dosing	
Patient Weight	**Induction/Loading Dosing**	**(A)**	**(B)**
Adults of any weight or any patient with a body weight that is greater than or equal to 40 kg	At least 900 (e.g., at least 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg once a week for four weeks	At least 1200 (e.g., at least 1225, 1250, 1300, 1350, or 1400 or more) mg on week five	Following the (A) dose, at least 1200 (e.g., at least 1225, 1250, 1300, 1350, or 1400 or more) mg once every two weeks thereafter*
Body weight that is less than 40 kg, but greater than or equal to 30 kg	At least 600 (e.g., at least 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, or 850 or more) mg once a week for two weeks	At least 900 (e.g., at least 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1050, 1100, 1150, or 1200 or more) mg on week three	Following the (A) dose, at least 900 (e.g., at least 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1050, 1100, 1150, or 1200 or more) mg once every two weeks thereafter*
Body weight that is less than 30 kg, but greater than or equal to 20 kg	At least 600 (e.g., at least 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, or 850 or more) mg once a week for two weeks	At least 600 (e.g., at least 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, or 850 or more) mg on week three	Following the (A) dose, at least 600 (e.g., at least 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, or 850 or more) mg once every two weeks thereafter*
Body weight that is less than 20 kg, but greater than or equal to 10 kg	At least 600 (e.g., at least 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, or 850 or more) mg once a week for one week	At least 300 (e.g., at least 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480,	Following the (A) dose, at least 300 (e.g., at least 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, or

		Maintenance Dosing	
Patient Weight	**Induction/Loading Dosing**	**(A)**	**(B)**
		490, 500, or 550 or more) mg on week two	550 or more) mg once every two weeks thereafter*
Body weight that is less than 10 kg, but greater than or equal to 5 kg	At least 300 (e.g., at least 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, or 550 or more) mg once a week for one week	At least 300 (e.g., at least 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, or 550 or more) mg on week two	Following the (A) dose, at least 300 (e.g., at least 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, or 550 or more) mg once every three weeks thereafter*

*In accordance with the present disclosure, the (B) maintenance dosing schedule can be maintained for the duration of the treatment regimen, e.g., at least one (e.g., at least two, three, four, five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 36, or 48 or more) month(s); at least one (e.g., at least two, three, four, five, six, seven, eight, nine, 10, 11, 12, 13, 14, or 15 or more) years; or for the remainder of the patient's life.

It is understood that the exemplary dosing schedules in Tables 1 or 2 can be adjusted (in frequency, duration, and/or in total amount of antibody administered) by a medical practitioner as necessary in such a way as to maintain complete or substantially complete inhibition of systemic complement activity in the patient for the duration of the dosing regime.

In another aspect, the disclosure features a method for treating a complement-associated disorder, the method including chronically administering to a patient in need thereof an anti-C5 antibody in an amount and with a frequency that are effective to maintain a concentration of at least 40 (e.g., at least 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 80, 85, 90, 95, 100, 110, or 120 or more) μg of the antibody per milliliter of the patient's blood, wherein the patient has, is suspected of having, or is at risk for

developing a complement-associated disorder with the proviso that the disorder is not paroxysmal nocturnal hemoglobinuria.

In some embodiments, the anti-C5 antibody is administered to the patient at least once every two weeks. In some embodiments, the anti-C5 antibody is administered to the patient once per week. In some embodiments, the anti-C5 antibody is administered to the patient for at least 9 weeks (e.g., 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 weeks; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, or 12 years or for the remainder of the patient's life) under the following dosing schedule: at least 800 (e.g., at least 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody, once per week for four consecutive weeks; at least 800 (e.g., at least 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody once during the fifth week; and at least 800 (e.g., at least 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody bi-weekly thereafter for the remainder of the dosing schedule. In preferred embodiments, at least 900 mg of the anti-C5 antibody is administered to the patient, once per week for four weeks; at least 1200 mg is administered to the patient during the fifth week; and at least 1200 mg of the anti-C5 antibody is administered to the patient bi-weekly thereafter for the remainder of the chronic dosing schedule.

In yet another aspect, the disclosure features a method for transplanting an organ or tissue. The method includes transplanting an organ or tissue into a patient in need thereof, wherein prior to and chronically following the transplanting an inhibitor of human complement is administered to the patient in an amount and with a frequency effective to substantially inhibit systemic complement activity in the patient. The complement inhibitor can be, e.g., a C5 inhibitor such as an anti-C5 antibody (e.g., eculizumab). As described herein, the C5 inhibitor (e.g., the anti-C5 antibody) can be administered in an amount and with a frequency to maintain a concentration of at least 0.7 bivalent C5 inhibitor molecule(s) (or at least 1.5 monovalent C5 inhibitor molecule(s)) per every C5 molecule in the patient's blood.

In some embodiments, the C5 inhibitor (e.g., the anti-C5 antibody) can be administered to the patient in an amount and with a frequency to maintain a concentration of at least at least 40 (e.g., 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 80, 85, 90, 95, 100, 110, or 120 or more) μg of the inhibitor (e.g., the anti-C5 antibody) in the patient's blood. In some embodiments, at least 800 (e.g., at least 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody (e.g., eculizumab) is administered to the patient less than 24 (e.g., less than 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or less than 2) hours prior to transplanting the organ or tissue to the patient. In some embodiments, the methods can also include, prior to the transplanting, contacting (e.g., soaking) the organ or tissue with a C5 inhibitor (e.g., an anti-C5 antibody such as eculizumab) for an amount of time and under conditions that inhibit complement activation in the organ or tissue upon transplantation. The organ can be, e.g., skin, a kidney, heart, lung, limb (e.g., finger or toe), eye, stem cell population, bone marrow, vascular tissue, muscle, nervous tissue, or liver. The patient can have, be at risk for developing, or be suspected of having aHUS. In some embodiments, at least 700 (e.g., at least 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of an anti-C5 antibody is administered to the patient less than 24 (e.g., less than 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or less than 2) hours following the transplanting. In some embodiments, the anti-C5 antibody is chronically administered to the patient following the transplanting. For example, an anti-C5 antibody can be chronically administered to the patient for at least 9 weeks (e.g., 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 weeks; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, or 12 years or for the remainder of the patient's life) under the following dosing schedule: at least 700 (e.g., at least 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody less than 24 hours after transplanting the organ or tissue; at least 700 (e.g., at least 710, 720,

730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody once per week for four weeks after the initial post-transplant dose; at least 700 (e.g., at least 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody once during the fifth week; and at least 700 (e.g., at least 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody bi-weekly thereafter for the remainder of the dosing schedule. In preferred embodiments, an anti-C5 antibody is administered to a patient undergoing a transplant operation under the following dosing schedule: at least 1200 mg of the anti-C5 antibody is administered to the patient less than 24 hours prior to the transplanting; at least 900 mg of the anti-C5 antibody is administered to the patient within 24 hours after the transplanting; at least 1200 mg of the anti-C5 antibody is administered to the patient once a week for four weeks following the first post-operation administration of the anti-C5 antibody; 1200 mg administered to the patient on the fifth week following the first post-operation administration of the anti-C5 antibody; and at least 1200 mg of the anti-C5 antibody administered to the patient bi-weekly thereafter for the remainder of the chronic treatment regimen.

In some embodiments, the methods can further include administering to the patient one or more immunosuppressive agents such as, but not limited to, rapamycin, cyclosporine A, an anti-IL-2 agent, OKT3, and tacrolimus. The one or more immunosuppressive agents can be administered prior to, during, or following the transplanting. The one or more immunosuppressive agents can also be administered before, concurrently with, or following administration of the C5 inhibitor.

The disclosure also features a method for reducing complement-mediated injury to an organ or a tissue when transplanted into a patient. The method includes, prior to transplanting an organ or tissue to a patient in need thereof, contacting the organ or tissue with a pharmaceutical solution comprising an inhibitor of C5 for a period of time and under conditions which reduce complement-mediated injury to the organ or tissue when transplanted into the patient. The C5 inhibitor can also be administered to the patient prior to, during, and/or after the transplanting of the organ

or tissue. The solution can also contain one or more immunosuppressive agents such as, but not limited to, rapamycin, cyclosporine A, an anti-IL-2 agent, OKT3, and tacrolimus.

The inventors have also discovered that in patients who have had severe complement-associated disorders such as CAPS and APS and entered remission, there still remains in the patients a low level of complement activity that predisposes the patients for relapse or recurrence. As noted above, recurrence of symptoms in patients who have had these severe disorders can present immediate and sometimes irreversible injury to major organs such as the kidney. Thus, while the disclosure is in no way limited by one particular theory or mechanism of action, the inventors assert that in order to prevent sudden relapse or recurrence, a patient with a complement-associated disorder (e.g., aHUS and CAPS) should be chronically treated with a C5 inhibitor even after one or more symptoms of the disorder have been ameliorated and/or even after the patient enters a clinical remission. Thus, in yet another aspect, the disclosure features a method for treating a complement-associated disorder with the proviso that the disorder is not paroxysmal nocturnal hemoglobinuria. The method includes administering to a patient afflicted with a complement-associated disorder a C5 inhibitor (e.g., an anti-C5 antibody such as eculizumab) in an amount effective to treat the complement-associated disorder. The C5 inhibitor is administered to the patient even after one or more (e.g., two, three, four, five, or six or more) symptoms of the disorder have been ameliorated. In some embodiments, the C5 inhibitor is administered to the patient even after one or more symptoms have been completely ameliorated. In some embodiments, the C5-inhibitor is administered to the patient even after the patient has entered clinical remission. The C5 inhibitor can be administered, e.g., chronically administered, to the patient for at least 8 weeks (e.g., 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 weeks; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, or 12 years or for the remainder of the patient's life) after one or more symptoms have been ameliorated in the patient and/or the patient enters clinical remission. The complement-associated disorder can be any of those described herein, e.g., membranoproliferative glomerulonephritis, Degos

disease, atypical hemolytic uremic syndrome, antibody-mediated rejection, HELLP syndrome, and catastrophic antiphospholipid syndrome.

While the disclosure is in no way limited by a particular theory or mechanism of action, based on the observations of the effect of eculizumab in, e.g., aHUS patients, the inventors have concluded that the biological activity of complement component C5a may substantially contribute to the vasoconstriction and hypertension associated with aHUS. Accordingly, inhibition of C5a using a C5a inhibitor is useful for treating aHUS and/or ameliorating the vasoconstriction and hypertension associated with aHUS. The method includes administering to a patient in need thereof an inhibitor of complement component C5a in an amount effective to treat aHUS in the patient. In some embodiments, the vasoconstriction and hypertension associated with aHUS can be ameliorated within less than two months (e.g., less than 7, 6, 5, 4, 3, or 2 weeks; less than 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 day(s); or less than 12, 11, 10, 9, 8, 7, 6 or even less than 5 hours) after administering the C5a inhibitor. In some embodiments, the C5a inhibitor is an antibody (or antigen-binding fragment thereof) that binds to a human C5a protein or a fragment thereof having an amino acid sequence that contains, or consists of, at least four (e.g., at least four, five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 16, or 17 or more) consecutive amino acids depicted in any one of SEQ ID NOs:12-25. In some embodiments, the inhibitor is an antibody that binds to human C5a protein having the amino acid sequence depicted in SEQ ID NO:12. The C5a inhibitor does not inhibit the cleavage of C5 into fragment C5a and C5b. The C5a inhibitor also does not inhibit C5b or the formation of the membrane attack complex. As described herein, in some embodiments, the C5a inhibitor (e.g., an anti-C5a antibody) can inhibit the interaction between C5a and a C5a receptor (e.g., C5aR or C5L2). In some embodiments, the antibody can be a monoclonal antibody, a single-chain antibody, a humanized antibody, a fully human antibody, a polyclonal antibody, a recombinant antibody, a diabody, a chimerized or chimeric antibody, a deimmunized human antibody, a fully human antibody, a single chain antibody, an Fv fragment, an Fd fragment, an Fab fragment, an Fab' fragment, or an $F(ab')_2$ fragment.

In embodiments of any of the methods described herein where the complement-associated disorder is aHUS, the aHUS can be genetic, acquired, or idiopathic form. In some embodiments, the aHUS can be complement factor H

(CFH)-associated aHUS (e.g., aHUS associated with mutations in factor H or autoantibodies that bind to and inactivate factor H), membrane cofactor protein (MCP)-associated aHUS, complement factor I (CFI)-associated aHUS, C4b-binding protein (C4BP)-associated aHUS, complement factor B-(CFB)-associated aHUS, a vWF disorder, or aHUS associated with any other mutations in the alternative pathway of complement activation causing low levels of C3 as a result of increased C3 consumption.

In some embodiments, any of the methods described herein can further include identifying the patient as one having, suspected of having, or at risk for developing, aHUS. In some embodiments, any of the methods described herein can include, after the administering, monitoring the patient for an improvement in one or more symptoms of aHUS. In some embodiments of any of the methods described herein, the C5a inhibitor can be administered to the patient prior to, during, or following a plasma therapy (e.g., plasma exchange or plasma infusion). In some embodiments, administration of the C5a inhibitor to the patient can alleviate the need for plasma therapy by a patient. For example, in some embodiments, administration (e.g., chronic administration) of the C5a inhibitor to the patient can alleviate or substantially reduce the need for plasma therapy by a patient for at least 2 months (e.g., 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 12 months or 1, 2, 3, 4, 5, or 6 years or more). In some embodiments, any of the methods described herein can include administering to the patient one or more additional active agents useful for treating typical HUS or aHUS. The one or more additional active agents can be, e.g., selected from the group consisting of anti-hypertensives, anti-platelet agents, prostacyclin, fibrinolytic agents, and anti-oxidants.

In some embodiments, the human is an infant. The infant can be, e.g., 0.5 (e.g., 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, or 9.5) years old. The infant can be less than 10 (e.g., less than 9.5, 9, 8.5, 8, 7.5, 7, 6.5, 6, 5.5, 5, 4.5, 4, 3.5, 3, 2.5, 2, 1.5, 1, or less than 1) year(s) old.

In some embodiments of any of the methods described herein, the complement-associated disorder is not paroxysmal nocturnal hemoglobinuria.

"Polypeptide," "peptide," and "protein" are used interchangeably and mean any peptide-linked chain of amino acids, regardless of length or post-translational modification. The complement component C5 proteins described herein can contain

or be wild-type proteins or can be variants that have not more than 50 (e.g., not more than one, two, three, four, five, six, seven, eight, nine, ten, 12, 15, 20, 25, 30, 35, 40, or 50) conservative amino acid substitutions. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine, glutamine, serine and threonine; lysine, histidine and arginine; and phenylalanine and tyrosine.

The human complement component C5 proteins described herein also include "antigenic peptide fragments" of the proteins, which are shorter than full-length and/or immature (pre-pro) C5 proteins, but retain at least 10% (e.g., at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, or 100% or more) of the ability of the full-length protein to induce an antigenic response in a mammal (see below under "Methods for Producing an Antibody"). Antigenic peptide fragments of a C5 protein include terminal as well internal deletion variants of the protein. Deletion variants can lack one, two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid segments (of two or more amino acids) or non-contiguous single amino acids. Antigenic peptide fragments can be at least 6 (e.g., at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, or 600 or more) amino acid residues in length (e.g., at least 6 contiguous amino acid residues in any one of SEQ ID NOS:1-11). In some embodiments, an antigenic peptide fragment of a human C5 protein has fewer than 500 (e.g., fewer than 450, 400, 350, 325, 300, 275, 250, 225, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, or 6) amino acid residues in length (e.g., fewer than 500 contiguous amino acid residues in any one of SEQ ID NOS:1-11). In some embodiments, an antigenic peptide fragment of a full-length, immature human C5 protein (prepro-C5 protein) has at least 6, but fewer than 500, amino acid residues in length.

In some embodiments, the human complement component C5 protein can have an amino acid sequence that is, or is greater than, 70 (e.g., 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100) % identical to the human C5 protein having the amino acid sequence depicted in SEQ ID NO:1 (see below).

Percent (%) amino acid sequence identity is defined as the percentage of amino acids in a candidate sequence that are identical to the amino acids in a reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent 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, ALIGN-2 or Megalign (DNASTAR) software. Appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared can be determined by known methods.

Amino acid sequences for exemplary human C5 proteins as well as antigenic peptide fragments thereof are known in the art and are set forth below.

As used throughout the present disclosure, the term "antibody" refers to a whole or intact antibody (e.g., IgM, IgG, IgA, IgD, or IgE) molecule that is generated by any one of a variety of methods that are known in the art and described herein. The term "antibody" includes a polyclonal antibody, a monoclonal antibody, a chimerized or chimeric antibody, a humanized antibody, a deimmunized human antibody, and a fully human antibody. The antibody can be made in or derived from any of a variety of species, e.g., mammals such as humans, non-human primates (e.g., monkeys, baboons, or chimpanzees), horses, cattle, pigs, sheep, goats, dogs, cats, rabbits, guinea pigs, gerbils, hamsters, rats, and mice. The antibody can be a purified or a recombinant antibody.

As used herein, the term "antibody fragment," "antigen-binding fragment," or similar terms refer to fragment of an antibody that retains the ability to bind to an antigen (e.g., a complement component C5 protein), e.g., a single chain antibody, a single chain Fv fragment (scFv), an Fd fragment, an Fab fragment, an Fab' fragment, or an $F(ab')_2$ fragment. An scFv fragment is a single polypeptide chain that includes both the heavy and light chain variable regions of the antibody from which the scFv is

derived. In addition, diabodies (Poljak (1994) *Structure* 2(12):1121-1123; Hudson et al. (1999) *J. Immunol. Methods* 23(1-2):177-189, the disclosures of each of which are incorporated herein by reference in their entirety) and intrabodies (Huston et al. (2001) *Hum. Antibodies* 10(3-4):127-142; Wheeler et al. (2003) *Mol Ther* 8(3):355-366; Stocks (2004) *Drug Discov. Today* 9(22): 960-966, the disclosures of each of which are incorporated herein by reference in their entirety) that bind to a complement component C5 protein can be incorporated into the compositions, and used in the methods, described herein.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the presently disclosed methods and compositions. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

Other features and advantages of the present disclosure, e.g., methods for treating or preventing a complement-associated disorder, will be apparent from the following description, the examples, and from the claims.

Detailed Description

The present disclosure provides compositions containing an inhibitor of human complement component C5 (e.g., an antibody that binds to a human complement component C5 protein or a biologically-active fragment thereof such as C5a and C5b) and methods for using the compositions to treat or prevent a complement-mediated disorder in a human. While in no way intended to be limiting, exemplary compositions (e.g., pharmaceutical compositions and formulations) and methods for using the compositions are elaborated on below.

Compositions

The compositions described herein contain an inhibitor of human complement. Any compound which binds to or otherwise blocks the generation and/or activity of any of the human complement components may be utilized in accordance with the

present disclosure. For example, an inhibitor of complement can be, e.g., a small molecule, a nucleic acid or nucleic acid analog, a peptidomimetic, or a macromolecule that is not a nucleic acid or a protein. These agents include, but are not limited to, small organic molecules, RNA aptamers, L-RNA aptamers, Spiegelmers, antisense compounds, double stranded RNA, small interfering RNA, locked nucleic acid inhibitors, and peptide nucleic acid inhibitors. In some embodiments, a complement inhibitor may be a protein or protein fragment.

In some embodiments, the compositions contain antibodies specific to a human complement component. Some compounds include antibodies directed against complement components C1, C2, C3, C4, C5 (or a fragment thereof; see below), C6, C7, C8, C9, Factor D, Factor B, Factor P, MBL, MASP-1, and MASP-2, thus preventing the generation of the anaphylatoxic activity associated with C5a and/or preventing the assembly of the membrane attack complex associated with C5b.

The compositions can also contain naturally occurring or soluble forms of complement inhibitory compounds such as CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH. Other compounds which may be utilized to bind to or otherwise block the generation and/or activity of any of the human complement components include, but are not limited to, proteins, protein fragments, peptides, small molecules, RNA aptamers including ARC187 (which is commercially available from Archemix Corporation, Cambridge, MA), L-RNA aptamers, spiegelmers, antisense compounds, serine protease inhibitors, molecules which may be utilized in RNA interference (RNAi) such as double stranded RNA including small interfering RNA (siRNA), locked nucleic acid (LNA) inhibitors, peptide nucleic acid (PNA) inhibitors, etc.

In some embodiments, the complement inhibitor inhibits the activation of complement. For example, the complement inhibitor can bind to and inhibit the complement activation activity of C1 (e.g., C1q, C1r, or C1s) or the complement inhibitor can bind to and inhibit (e.g., inhibit cleavage of) C2, C3, or C4. In some embodiments, the inhibitor inhibits formation or assembly of the C3 convertase and/or C5 convertase of the alternative and/or classical pathways of complement. In some embodiments, the complement inhibitor inhibits terminal complement formation, e.g., formation of the C5b-9 membrane attack complex. For example, an antibody complement inhibitor may include an anti-C5 antibody. Such anti-C5 antibodies may

directly interact with C5 and/or C5b, so as to inhibit the formation of and/or physiologic function of C5b.

In some embodiments, the compositions described herein can contain an inhibitor of human complement component C5 (e.g., an antibody, or antigen-binding fragment thereof, that binds to a human complement component C5 protein or a biologically-active fragment thereof such as C5a or C5b). As used herein, an "inhibitor of complement component C5" is any agent that inhibits: (i) the expression, or proper intracellular trafficking or secretion by a cell, of a complement component C5 protein; (ii) the activity of C5 cleavage fragments C5a or C5b (e.g., the binding of C5a to its cognate cellular receptors or the binding of C5b to C6 and/or other components of the terminal complement complex; see above); (iii) the cleavage of a human C5 protein to form C5a and C5b; or (iv) the proper intracellular trafficking of, or secretion by a cell, of a complement component C5 protein. Inhibition of complement component C5 protein expression includes: inhibition of transcription of a gene encoding a human C5 protein; increased degradation of an mRNA encoding a human C5 protein; inhibition of translation of an mRNA encoding a human C5 protein; increased degradation of a human C5 protein; inhibition of proper processing of a pre-pro human C5 protein; or inhibition of proper trafficking or secretion by a cell of a human C5 protein. Methods for determining whether a candidate agent is an inhibitor of human complement component C5 are known in the art and described herein.

An inhibitor of human complement component C5 can be, e.g., a small molecule, a polypeptide, a polypeptide analog, a nucleic acid, or a nucleic acid analog.

"Small molecule" as used herein, is meant to refer to an agent, which has a molecular weight of less than about 6 kDa and most preferably less than about 2.5 kDa. Many pharmaceutical companies have extensive libraries of chemical and/or biological mixtures comprising arrays of small molecules, often fungal, bacterial, or algal extracts, which can be screened with any of the assays of the application. This application contemplates using, among other things, small chemical libraries, peptide libraries, or collections of natural products. Tan et al. described a library with over two million synthetic compounds that is compatible with miniaturized cell-based assays (*J. Am. Chem. Soc.* (1998) 120:8565-8566). It is within the scope of this

application that such a library may be used to screen for inhibitors of human complement component C5. There are numerous commercially available compound libraries, such as the Chembridge DIVERSet. Libraries are also available from academic investigators, such as the Diversity set from the NCI developmental therapeutics program. Rational drug design may also be employed. For example, rational drug design can employ the use of crystal or solution structural information on the human complement component C5 protein. See, e.g., the structures described in Hagemann et al. (2008) *J Biol Chem* 283(12):7763-75 and Zuiderweg et al. (1989) *Biochemistry* 28(1):172–85. Rational drug design can also be achieved based on known compounds, e.g., a known inhibitor of C5 (e.g., an antibody, or antigen-binding fragment thereof, that binds to a human complement component C5 protein).

Peptidomimetics can be compounds in which at least a portion of a subject polypeptide is modified, and the three dimensional structure of the peptidomimetic remains substantially the same as that of the subject polypeptide. Peptidomimetics may be analogues of a subject polypeptide of the disclosure that are, themselves, polypeptides containing one or more substitutions or other modifications within the subject polypeptide sequence. Alternatively, at least a portion of the subject polypeptide sequence may be replaced with a non-peptide structure, such that the three-dimensional structure of the subject polypeptide is substantially retained. In other words, one, two or three amino acid residues within the subject polypeptide sequence may be replaced by a non-peptide structure. In addition, other peptide portions of the subject polypeptide may, but need not, be replaced with a non-peptide structure. Peptidomimetics (both peptide and non-peptidyl analogues) may have improved properties (e.g., decreased proteolysis, increased retention or increased bioavailability). Peptidomimetics generally have improved oral availability, which makes them especially suited to treatment of disorders in a human or animal. It should be noted that peptidomimetics may or may not have similar two-dimensional chemical structures, but share common three-dimensional structural features and geometry. Each peptidomimetic may further have one or more unique additional binding elements.

Nucleic acid inhibitors can be used to decrease expression of an endogenous gene encoding human complement component C5. The nucleic acid antagonist can be, e.g., an siRNA, a dsRNA, a ribozyme, a triple-helix former, an aptamer, or an

antisense nucleic acid. siRNAs are small double stranded RNAs (dsRNAs) that optionally include overhangs. For example, the duplex region of an siRNA is about 18 to 25 nucleotides in length, e.g., about 19, 20, 21, 22, 23, or 24 nucleotides in length. The siRNA sequences can be, in some embodiments, exactly complementary to the target mRNA. dsRNAs and siRNAs in particular can be used to silence gene expression in mammalian cells (e.g., human cells). See, e.g., Clemens et al. (2000) *Proc. Natl. Acad. Sci. USA* 97:6499- 6503; Billy et al. (2001) *Proc. Natl. Acad. Sci. USA* 98:14428-14433; Elbashir et al. (2001) *Nature* 411 :494-8; Yang et al. (2002) *Proc. Natl. Acad. Sci. USA* 99:9942-9947, and U.S. Patent Application Publication Nos. 20030166282, 20030143204, 20040038278, and 20030224432. Anti-sense agents can include, for example, from about 8 to about 80 nucleobases (i.e. from about 8 to about 80 nucleotides), e.g., about 8 to about 50 nucleobases, or about 12 to about 30 nucleobases. Anti-sense compounds include ribozymes, external guide sequence (EGS) oligonucleotides (oligozymes), and other short catalytic RNAs or catalytic oligonucleotides which hybridize to the target nucleic acid and modulate its expression. Anti-sense compounds can include a stretch of at least eight consecutive nucleobases that are complementary to a sequence in the target gene. An oligonucleotide need not be 100% complementary to its target nucleic acid sequence to be specifically hybridizable. An oligonucleotide is specifically hybridizable when binding of the oligonucleotide to the target interferes with the normal function of the target molecule to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment or, in the case of *in vitro* assays, under conditions in which the assays are conducted. Hybridization of antisense oligonucleotides with mRNA (e.g., an mRNA encoding a human C5 protein) can interfere with one or more of the normal functions of mRNA. The functions of mRNA to be interfered with include all key functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in by the RNA. Binding of specific protein(s) to the RNA may also be interfered with by antisense oligonucleotide hybridization to the RNA. Exemplary antisense compounds include DNA or RNA

sequences that specifically hybridize to the target nucleic acid, e.g., the mRNA encoding a human complement component C5 protein. The complementary region can extend for between about 8 to about 80 nucleobases. The compounds can include one or more modified nucleobases.

Modified nucleobases may include, e.g., 5-substituted pyrimidines such as 5-iodouracil, 5-iodocytosine, and C_5- propynyl pyrimidines such as C_5-propynylcytosine and C_5-propynyluracil. Other suitable modified nucleobases include, e.g., 7-substituted- 8-aza-7-deazapurines and 7-substituted-7-deazapurines such as, for example, 7-iodo-7- deazapurines, 7-cyano-7-deazapurines, 7-aminocarbonyl-7-deazapurines. Examples of these include 6-amino-7-iodo-7-deazapurines, 6-amino-7-cyano-7-deazapurines, 6- amino-7-aminocarbonyl-7-deazapurines, 2-amino-6-hydroxy-7-iodo-7-deazapurines, 2- amino-6-hydroxy-7-cyano-7-deazapurines, and 2-amino-6-hydroxy-7-aminocarbonyl-7-deazapurines. See, e.g., U.S. Patent Nos. 4,987,071; 5,116,742; and 5,093,246; "Antisense RNA and DNA," D.A. Melton, Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1988); Haselhoff and Gerlach (1988) *Nature* 334:585-59; Helene, C. (1991) *Anticancer Drug D* 6:569-84; Helene (1992) *Ann. NY. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14:807-15.

Aptamers are short oligonucleotide sequences that can be used to recognize and specifically bind almost any molecule, including cell surface proteins. The systematic evolution of ligands by exponential enrichment (SELEX) process is powerful and can be used to readily identify such aptamers. Aptamers can be made for a wide range of proteins of importance for therapy and diagnostics, such as growth factors and cell surface antigens. These oligonucleotides bind their targets with similar affinities and specificities as antibodies do (see, e.g., Ulrich (2006) *Handb Exp Pharmacol.* 173:305-326).

In some embodiments, the inhibitor of human C5 is an antibody, or antigen-binding fragment thereof, which binds to a human complement component C5 protein. (Hereinafter, the antibody may sometimes be referred to as an "anti-C5 antibody.")

In some embodiments, the anti-C5 antibody binds to an epitope in the human pro-C5 precursor protein. For example, the anti-C5 antibody can bind to an epitope in the human complement component C5 protein comprising, or consisting of, the amino

acid sequence depicted in SEQ ID NO:1 (NCBI Accession No. AAA51925 and Haviland et al., *supra*).

An "epitope" refers to the site on a protein (e.g., a human complement component C5 protein) that is bound by an antibody. "Overlapping epitopes" include at least one (e.g., two, three, four, five, or six) common amino acid residue(s).

In some embodiments, the anti-C5 antibody binds to an epitope in the human pro-C5 precursor protein lacking the leader sequence. For example, the anti-C5 antibody can bind to an epitope in the human complement component C5 protein comprising, or consisting of, the amino acid sequence depicted in SEQ ID NO:2, which is a human C5 protein lacking the amino terminal leader sequence.

In some embodiments, the anti-C5 antibody can bind to an epitope in the alpha chain of the human complement component C5 protein. For example, the anti-C5 antibody can bind to an epitope within, or overlapping with, a protein having the amino acid sequence depicted in SEQ ID NO:3, which is the human complement component C5 alpha chain protein. Antibodies that bind to the alpha chain of C5 are described in, for example, Ames et al. (1994) *J Immunol* 152:4572-4581.

In some embodiments, the anti-C5 antibody can bind to an epitope in the beta chain of the human complement component C5 protein. For example, the anti-C5 antibody can bind to an epitope within, or overlapping with, a protein having the amino acid sequence depicted in SEQ ID NO:4, which is the human complement component C5 beta chain protein. Antibodies that bind to the C5 beta chain are described in, e.g., Moongkarndi et al. (1982) *Immunobiol.* 162:397; Moongkarndi et al. (1983) *Immunobiol.* 165:323; and Mollnes et al. (1988) *Scand. J. Immunol.* 28:307-312.

In some embodiments, the anti-C5 antibody can bind to an epitope within, or overlapping with, an antigenic peptide fragment of a human complement component C5 protein. For example, the anti-C5 antibody can bind to an epitope within, or overlapping with, an antigen peptide fragment of a human complement component C5 protein, the fragment containing, or consisting of, the following amino acid sequence: VIDHQGTKSSKCVRQKVEGSS (SEQ ID NO:5) or KSSKC (SEQ ID NO:6).

In some embodiments, the anti-C5 antibody can bind to an epitope within, or overlapping with, a fragment of a human complement component C5 protein, the fragment containing, or consisting of, any one of the following amino acid sequences

(which are exemplary antigenic fragments of SEQ ID NO:1):
NFSLETWFGKEILVKTLRVVPEGVKRESYSGVTLDPRGIYGTISRRKEFPYRIP
LDLVPKTEIKRILSVKGLLVGEILSAVLSQEGINILTHLPKGSAEAELMSVVPVF
YVFHYLETGNHWNIFHSD (SEQ ID NO:7);
SESPVIDHQGTKSSKCVRQKVEGSSSHLVTFTVLPLEIGLHNINFSLETWFGKEI
LVKTLRVVPEGVKRESYSGVTLDPRGIYGTISRRKEFPYRIPLDLVPKTEIKRIL
SVKGLLVGEILSAVLSQEGINILTHLPKGSAEAELMSVVPVFYVFHYLETGNH
WNIFHSDPLIEKQKLKKKLKEGMLSIMSYRNADYSYS (SEQ ID NO:8);
SHKDMQLGRLHMKTLLPVSKPEIRSYFPES (SEQ ID NO:9);
SHKDMQLGRLHMKTLLPVSKPEIRSYFPESWLWEVHLVPRRKQLQFALPDSL
TTWEIQGIGISNTGICVADTVKAKVFKDVFLEMNIPYSVVRGEQIQLKGTVYN
YRTSGMQFCVKMSAVEGICTSESPVIDHQGTKSSKCVRQKVEGSSSHLVTFTV
LPLEIGLHNINFSLETWFGKEILVKTLRVVPEGVKRESYSGVTLDPRGIYGTISR
RKEFPYRIPLDLVPKTEIKRILSVKGLLVGEILSAVLSQEGINILTHLPKGSAEAE
LMSVVPVFYVFHYLETGNHWNIFHSDPLIEKQKLKKKLKEGMLSIMSYRNAD
YSYS (SEQ ID NO:10); and
DHQGTKSSKCVRQKVEG (SEQ ID NO:11).

Additional exemplary antigenic fragments of human complement component C5 are disclosed in, e.g., U.S. Patent No. 6,355,245, the disclosure of which is incorporated herein by reference.

In some embodiments, the anti-C5 antibody specifically binds to a human complement component C5 protein (e.g., the human C5 protein having the amino acid sequence depicted in SEQ ID NO:1). The terms "specific binding" or "specifically binds" refer to two molecules forming a complex (e.g., a complex between an antibody and a complement component C5 protein) that is relatively stable under physiologic conditions. Typically, binding is considered specific when the association constant (K_a) is higher than 10^6 M^{-1}. Thus, an antibody can specifically bind to a C5 protein with a K_a of at least (or greater than) 10^6 (e.g., at least or greater than 10^7, 10^8, 10^9, 10^{10}, 10^{11} 10^{12}, 10^{13}, 10^{14}, or 10^{15} or higher) M^{-1}. Examples of antibodies that specifically bind to a human complement component C5 protein are described in, e.g., U.S. Patent No. 6,355,245, the disclosure of which is incorporated herein by reference in its entirety.

Methods for determining whether an antibody binds to a protein antigen and/or the affinity for an antibody to a protein antigen are known in the art. For example, the binding of an antibody to a protein antigen can be detected and/or quantified using a variety of techniques such as, but not limited to, Western blot, dot blot, plasmon surface resonance method (e.g., BIAcore system; Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.), or enzyme-linked immunosorbent assays (ELISA). See, e.g., Harlow and Lane (1988) "Antibodies: A Laboratory Manual" Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Benny K. C. Lo (2004) "Antibody Engineering: Methods and Protocols," Humana Press (ISBN: 1588290921); Borrebaek (1992) "Antibody Engineering, A Practical Guide," W.H. Freeman and Co., NY; Borrebaek (1995) "Antibody Engineering," 2nd Edition, Oxford University Press, NY, Oxford; Johne et al. (1993) *J. Immunol. Meth.* 160:191-198; Jonsson et al. (1993) *Ann. Biol. Clin.* 51:19-26; and Jonsson et al. (1991) *Biotechniques* 11:620-627. See also, U.S. Patent No. 6,355,245.

In some embodiments, the anti-C5 antibody can crossblock binding of another antibody that binds to an epitope within, or overlapping with, a human complement component C5 protein. In some embodiments, the anti-C5 antibody can crossblock binding of an antibody that binds to an epitope within, or overlapping with, a peptide fragment of a human complement component C5 protein. The peptide fragment can be a fragment of a human complement component C5 protein having the amino acid sequence depicted in any one of SEQ ID NOS:1-11. For example, the peptide fragment can contain, or consist of, the following amino acid sequence: VIDHQGTKSSKCVRQKVEGSS (SEQ ID NO:5).

As used herein, the term "crossblocking antibody" refers to an antibody that lowers the amount of binding of anti-C5 antibody to an epitope on a complement component C5 protein relative to the amount of binding of the anti-C5 antibody to the epitope in the absence of the antibody. Suitable methods for determining whether a first antibody crossblocks binding of a second antibody to an epitope are known in the art. For example, crossblocking antibodies can be identified by comparing the binding of the 5G1.1 anti-C5 monoclonal antibody (produced by the hybridoma cell line ATCC designation HB-11625; see U.S. Patent No. 6,355,245) in the presence and absence of a test antibody. Decreased binding of the 5G1.1 antibody in the presence

of the test antibody as compared to binding of the 5G1.1 antibody in the absence of the test antibody indicates the test antibody is a crossblocking antibody.

Methods for identifying the epitope to which a particular antibody (e.g., an anti-C5 antibody) binds are also known in the art. For example, the binding epitope of an anti-C5 antibody can be identified by measuring the binding of the antibody to several (e.g., three, four, five, six, seven, eight, nine, 10, 15, 20, or 30 or more) overlapping peptide fragments of a complement component C5 protein (e.g., several overlapping fragments of a protein having the amino acid sequence depicted in any one of SEQ ID NOs:1-11). Each of the different overlapping peptides is then bound to a unique address on a solid support, e.g., separate wells of a multi-well assay plate. Next, the anti-C5 antibody is interrogated by contacting it to each of the peptides in the assay plate for an amount of time and under conditions that allow for the antibody to bind to its epitope. Unbound anti-C5 antibody is removed by washing each of the wells. Next, a detectably-labeled secondary antibody that binds to the anti-C5 antibody, if present in a well of the plate, is contacted to each of the wells, and unbound secondary antibody is removed by washing steps. The presence or amount of the detectable signal produced by the detectably-labeled secondary antibody in a well is an indication that the anti-C5 antibody binds to the particular peptide fragment associated with the well. See, e.g., Harlow and Lane (*supra*), Benny K. C. Lo (*supra*), and U.S. Patent Application Publication No. 20060153836, the disclosure of which is incorporated by reference in its entirety. A particular epitope to which an antibody binds can also be identified using BIAcore chromatographic techniques (see, e.g., Pharmacia BIAtechnology Handbook, "Epitope Mapping," Section 6.3.2, (May 1994); and Johne et al. (1993) *J. Immunol. Methods* 160:20191-8).

The anti-C5 antibodies described herein can have activity in blocking the generation or activity of the C5a and/or C5b active fragments of a complement component C5 protein (e.g., a human C5 protein). Through this blocking effect, the anti-C5 antibodies inhibit, e.g., the proinflammatory effects of C5a and the generation of the C5b-9 membrane attack complex (MAC) at the surface of a cell. Anti-C5 antibodies that have the ability to block the generation of C5a are described in, e.g., Moongkarndi et al. (1982) *Immunobiol.* 162:397 and Moongkarndi et al. (1983) *Immunobiol.* 165:323.

In some embodiments, an anti-C5 antibody, or antigen-binding fragment thereof, can reduce the ability of a C5 protein to bind to human complement component C3b (e.g., C3b present in an AP or CP C5 convertase complex) by greater than 50 (e.g., greater than 55, 60, 65, 70, 75, 80, 85, 90, or 95 or more) %. In some embodiments, upon binding to a C5 protein, the anti-C5 antibody or antigen-binding fragment thereof can reduce the ability of the C5 protein to bind to complement component C4b (e.g., C4b present in a CP C5 convertase) by greater than 50 (e.g., greater than 55, 60, 65, 70, 75, 80, 85, 90, or 95 or more) %. Methods for determining whether an antibody can block the generation or activity of the C5a and/or C5b active fragments of a complement component C5 protein, or binding to complement component C4b or C3b, are known in the art and described in, e.g., U.S. Patent No. 6,355,245 and Wurzner et al. (1991) *Complement Inflamm* 8:328-340. (See also below.)

In some embodiments, an anti-C5 antibody binds to an amino-terminal region of the alpha chain of a complement component C5 protein, but does not bind to free C5a. Epitopes for an anti-C5 antibody within the amino-terminal region of the alpha chain include, e.g., epitopes within the human sequence VIDHQGTKSSKCVRQKVEGSS (SEQ ID NO:5).

In some embodiments, the composition comprises, and/or the antibody is, eculizumab (Soliris®; Alexion Pharmaceuticals, Inc., Cheshire, CT). (See, e.g., Kaplan (2002) *Curr Opin Investig Drugs* 3(7):1017-23; Hill (2005) *Clin Adv Hematol Oncol* 3(11):849-50; and Rother et al. (2007) *Nature Biotechnology* 25(11):1256-1488.)

In some embodiments, the composition comprises, and/or the antibody is, pexelizumab (Alexion Pharmaceuticals, Inc., Cheshire, CT). (See, e.g., Whiss (2002) *Curr Opin Investig Drugs* 3(6):870-7; Patel et al. (2005) *Drugs Today (Barc)* 41(3):165-70; and Thomas et al. (1996) *Mol Immunol.* 33(17-18):1389-401.)

In some embodiments, the C5 inhibitor is an antibody that binds to C5a (sometimes referred to herein as "an anti-C5a antibody"). In some embodiments, the antibody binds to C5a, but not to full-length C5. As discussed above, the proform of C5, a 1676 amino acid residue precursor protein, is processed by a series of proteolytic cleavage events. The first 18 peptides (numbered -18 to -1) constitute a signal peptide that is cleaved from the precursor protein. The remaining 1658 amino

acid protein is cleaved in two places to form the alpha and beta chains. The first cleavage event occurs between amino acid residues 655 and 656. The second cleavage occurs between amino acid residues 659 to 660. The two cleavage events result in the formation of three distinct polypeptide fragments: (i) a fragment comprising amino acids 1 to 655, which is referred to as the beta chain; (ii) a fragment comprising amino acids 660 to 1658, which is referred to as the alpha chain; and (iii) a tetrapeptide fragment consisting of amino acids 656 to 659. The alpha chain and the beta chain polypeptide fragments are connected to each other via disulfide bond and constitute the mature C5 protein. The CP or AP C5 convertase activates mature C5 by cleaving the alpha chain between residues 733 and 734, which results in the liberation of C5a fragment (amino acids 660 to 733). The remaining portion of mature C5 is fragment C5b, which contains the residues 734 to 1658 of the alpha chain disulfide bonded to the beta chain.

In vivo, C5a is rapidly metabolized by a serum enzyme, carboxypeptidase B, to a 73 amino acid form termed "C5a des-Arg," which has lost the carboxyterminal arginine residue. Accordingly, in some embodiments, an antibody that binds to C5a also binds to desarginated C5a. In some embodiments, an antibody that binds to C5a does not bind to desarginated C5a.

In some embodiments, the C5 inhibitor is an antibody that binds to a neoepitope present in C5a, i.e., an epitope that becomes exposed upon the liberation of C5a from the alpha chain fragment of mature C5. Antibodies that bind to C5a (e.g., a neo-epitope present in C5a) are known in the art as are methods for producing such antibodies. For example, an antibody that binds to C5a can have the binding specificity of a C5a neoepitope specific antibody described in any one of, e.g., PCT Publication No. WO 01/15731; Ames et al. (1994) *J Immunol* 152(9):4572-4581; Inoue (1989) *Complement Inflamm* 6(3):219-222; and U.S. Patent No. 6,866,845. In another example, an antibody that binds to C5a can have the binding specificity of a commercial C5a neoepitope-specific antibody such as, but not limited to, sc-52633 (Santa Cruz Biotechnology, Inc., Santa Cruz, California), I52-1486 (BD Pharmingen/BD Biosciences), ab11877 (Abcam, Cambridge, Massachusetts), and HM2079 (clone 2952; HyCult Biotechnology, the Netherlands). In some embodiments, an antibody that binds to C5a can crossblock the binding of any of the aforementioned C5a neoepitope-specific antibodies.

In some embodiments, the C5 inhibitor can be an antibody that binds to a mammalian (e.g., human) C5a protein. For example, the antibody can bind to a human C5a protein having the following amino acid sequence: TLQKKIEEIAAKYKHSVVKKCCYDGACVNNDETCEQRAARISLGPRCIKAFTE CCVVASQLRANISHKDMQLGR (SEQ ID NO:12). The antibody can bind to human C5a at an epitope within or overlapping with the amino acid sequence: CCYDGACVNNDETCEQRAAR (SEQ ID NO:13); KCCYDGACVNNDETCEQR (SEQ ID NO:14); VNNDETCEQR (SEQ ID NO:15); VNNDET (SEQ ID NO:16); AARISLGPR (SEQ ID NO:17); CCYDGACVNNDETCEQRAA (SEQ ID NO:18); CCYDGACVNNDETCEQRA (SEQ ID NO:19); CCYDGACVNNDETCEQR (SEQ ID NO:20); CCYDGACVNNDETCEQ (SEQ ID NO:21); CCYDGACVNNDETCE (SEQ ID NO:22); CYDGACVNNDETCEQRAAR (SEQ ID NO:23); YDGACVNNDETCEQRAAR (SEQ ID NO:24); or CYDGACVNNDETCEQRAAR (SEQ ID NO:25). In some embodiments, an antibody can bind to a human C5a protein or fragment thereof containing an amino acid sequence that contains, or consists of, at least four (e.g., at least four, five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 16, or 17 or more) consecutive amino acids depicted in any one of SEQ ID NOs:12-25. Additional C5a protein fragments to which an antibody described herein can bind and methods for generating suitable C5a-specific antigen combining sites are set forth in, e.g., U.S. Patent No. 4,686,100, the disclosure of which is incorporated herein by reference in its entirety.

In some embodiments, the binding of an antibody to C5a can inhibit the biological activity of C5a. Methods for measuring C5a activity include, e.g., chemotaxis assays, RIAs, or ELISAs (see, e.g., Ward and Zvaifler (1971) *J Clin Invest* 50(3):606-16 and Wurzner et al. (1991) *Complement Inflamm* 8:328-340). In some embodiments, the binding of an antibody to C5a can inhibit the interaction between C5a and C5aR1. Suitable methods for detecting and/or measuring the interaction between C5a and C5aR1 (in the presence and absence of an antibody) are known in the art and described in, e.g., Mary and Boulay (1993) *Eur J Haematol* 51(5):282-287; Kaneko et al. (1995) *Immunology* 86(1):149-154; Giannini et al. (1995) *J Biol Chem* 270(32):19166-19172; and U.S. Patent Application Publication No. 20060160726. For example, the binding of detectably labeled (e.g., radioactively labeled) C5a to C5aR1-expressing peripheral blood mononuclear cells can be

evaluated in the presence and absence of an antibody. A decrease in the amount of detectably-labeled C5a that binds to C5aR1 in the presence of the antibody, as compared to the amount of binding in the absence of the antibody, is an indication that the antibody inhibits the interaction between C5a and C5aR1. In some embodiments, the binding of an antibody to C5a can inhibit the interaction between C5a and C5L2 (see below). Methods for detecting and/or measuring the interaction between C5a and C5L2 are known in the art and described in, e.g., Ward (2009) *J Mol Med* 87(4):375-378 and Chen et al. (2007) *Nature* 446(7132):203-207 (see below).

In some embodiments, the C5 inhibitor is an antibody that binds to C5b (sometimes referred to herein as "an anti-C5b antibody"). In some embodiments, the antibody binds to C5b, but does not bind to full-length C5. The structure of C5b is described above and also detailed in, e.g., Müller-Eberhard (1985) *Biochem Soc Symp* 50:235-246; Yamamoto and Gewurz (1978) *J Immunol* 120(6):2008-2015; and Haviland et al. (1991), *supra*. As described above, C5b combines with C6, C7, and C8 to form the C5b-8 complex at the surface of the target cell. Protein complex intermediates formed during the series of combinations include C5b-6 (including C5b and C6), C5b-7 (including C5b, C6, and C7), and C5b-8 (including C5b, C6, C7, and C8). Upon binding of several C9 molecules, the membrane attack complex (MAC, C5b-9 terminal complement complex (TCC)) is formed. When sufficient numbers of MACs insert into target cell membranes, the openings they create (MAC pores) mediate rapid osmotic lysis of the target cells.

In some embodiments, the binding of an antibody to C5b can inhibit the interaction between C5b and C6. In some embodiments, the binding of the antibody to C5b can inhibit the assembly or activity of the C5b-9 MAC-TCC. In some embodiments, the binding of an antibody to C5b can inhibit complement-dependent cell lysis (e.g., *in vitro* and/or *in vivo*). Suitable methods for evaluating whether an antibody inhibits complement-dependent lysis include, e.g., hemolytic assays or other functional assays for detecting the activity of soluble C5b-9. For example, a reduction in the cell-lysing ability of complement in the presence of an antibody can be measured by a hemolysis assay described by Kabat and Mayer (eds.), "Experimental Immunochemistry, 2nd Edition," 135-240, Springfield, IL, CC Thomas (1961), pages 135-139, or a conventional variation of that assay such as the chicken

erythrocyte hemolysis method as described in, e.g., Hillmen et al. (2004) *N Engl J Med* 350(6):552.

Antibodies that bind to C5b as well as methods for making such antibodies are known in the art. See, e.g., U.S. Patent No. 6,355,245. Commercially available anti-C5b antibodies are available from a number of vendors including, e.g., Hycult Biotechnology (catalogue number: HM2080; clone 568) and Abcam™ (ab46151 or ab46168).

In some embodiments, the C5 inhibitor is an antibody that binds to a mammalian (e.g., human) form of C5b. For example, the antibody can bind to a portion of a human C5b protein having the following amino acid sequence: QEQTYVISAPKIFRVGASENIVIQVYGYTEAFDATISIKSYPDKKFSYSSGHVHL SSENKFQNSAILTIQPKQLPGGQNPVSYVYLEVVSKHFSKSKRMPITYDNGFLF IHTDKPVYTPDQSVKVRVYSLNDDLKPAKRETVLTFIDPEGSEVDMVEEIDHI GIISFPDFKIPSNPRYGMWTIKAKYKEDFSTTGTAYFEVKEYVLPHFSVSIEPEY NFIGYKNFKNFEITIKARYFYNKVVTEADVYITFGIREDLKDDQKEMMQTAM QNTMLINGIAQVTFDSETAVKELSYYSLEDLNNKYLYIAVTVIESTGGFSEEAE IPGIKYVLSPYKLNLVATPLFLKPGIPYPIKVQVKDSLDQLVGGVPVILNAQTID VNQETSDLDPSKSVTRVDDGVASFVLNLPSGVTVLEFNVKTDAPDLPEENQA REGYRAIAYSSLSQSYLYIDWTDNHKALLVGEHLNIIVTPKSPYIDKITHYNYL ILSKGKIIHFGTREKFSDASYQSINIPVTQNMVPSSRLLVYYIVTGEQTAELVSD SVWLNIEEKCGNQLQVHLSPDADAYSPGQTVSLNMATGMDSWVALAAVDS AVYGVQRGAKKPLERVFQFLEKSDLGCGAGGGLNNANVFHLAGLTFLTNAN ADDSQENDEPCKEIL (SEQ ID NO:4). In some embodiments, the antibody can bind to a portion of a human C5b protein having the following amino acid sequence: LHMKTLLPVSKPEIRSYFPESWLWEVHLVPRRKQLQFALPDSLTTWEIQGIGIS NTGICVADTVKAKVFKDVFLEMNIPYSVVRGEQIQLKGTVYNYRTSGMQFCV KMSAVEGICTSESPVIDHQGTKSSKCVRQKVEGSSSHLVTFTVLPLEIGLHNIN FSLETWFGKEILVKTLRVVPEGVKRESYSGVTLDPRGIYGTISRRKEFPYRIPL DLVPKTEIKRILSVKGLLVGEILSAVLSQEGINILTHLPKGSAEAELMSVVPVFY VFHYLETGNHWNIFHSDPLIEKQKLKKKLKEGMLSIMSYRNADYSYSVWKG GSASTWLTAFALRVLGQVNKYVEQNQNSICNSLLWLVENYQLDNGSFKENS QYQPIKLQGTLPVEARENSLYLTAFTVIGIRKAFDICPLVKIDTALIKADNFLLE NTLPAQSTFTLAISAYALSLGDKTHPQFRSIVSALKREALVKGNPPIYRFWKD

NLQHKDSSVPNTGTARMVETTAYALLTSLNLKDINYVNPVIKWLSEEQRYGG
GFYSTQDTINAIEGLTEYSLLVKQLRLSMDIDVSYKHKGALHNYKMTDKNFL
GRPVEVLLNDDLIVSTGFGSGLATVHVTTVVHKTSTSEEVCSFYLKIDTQDIEA
SHYRGYGNSDYKRIVACASYKPSREESSSGSSHAVMDISLPTGISANEEDLKA
LVEGVDQLFTDYQIKDGHVILQLNSIPSS
DFLCVRFRIFELFEVGFLSPATFTVYEYHRPDKQCTMFYSTSNIKIQKVCEGAA
CKCVEADCGQMQEELDLTISAETRKQTACKPEIAYAYKVSITSITVENVFVKY
KATLLDIYKTGEAVAEKDSEITFIKKVTCTNAELVKGRQYLIMGKEALQIKYN
FSFRYIYPLDSLTWIEYWPRDTTCSSCQAFLANLDEFAEDIFLNGC (SEQ ID NO:26). In some embodiments, the antibody can bind to human C5b protein or fragment thereof containing an amino acid sequence that contains, or consists of, at least four (e.g., at least four, five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more) consecutive amino acids depicted in SEQ ID NO:4 or SEQ ID NO:26.

Additional exemplary sub-fragments of human C5b or C5a to which a C5 inhibitor antibody can bind are disclosed in, e.g., U.S. Patent No. 6,355,245, the disclosure of which is incorporated herein by reference.

In some embodiments, the inhibitor is an antibody that specifically binds to a C5a polypeptide (e.g., the human C5a polypeptide having the amino acid sequence depicted in SEQ ID NO:12). In some embodiments, the inhibitor is an antibody that specifically binds to a C5b polypeptide.

Methods for determining whether a particular agent is an inhibitor of human complement component C5 are described herein and are known in the art. For example, the concentration and/or physiologic activity of C5a and C5b in a body fluid can be measured by methods well known in the art. Methods for measuring C5a concentration or activity include, e.g., chemotaxis assays, RIAs, or ELISAs (see, e.g., Ward and Zvaifler (1971) *J Clin Invest.* 50(3):606-16 and Wurzner et al. (1991) *Complement Inflamm.* 8:328-340). For C5b, hemolytic assays or assays for soluble C5b-9 as discussed herein can be used. Other assays known in the art can also be used. Using assays of these or other suitable types, candidate agents capable of inhibiting human complement component C5 such as an anti-C5 antibody, can be screened in order to, e.g., identify compounds that are useful in the methods described herein and determine the appropriate dosage levels of such compounds.

Methods for detecting inhibition of expression of mRNA or protein (e.g., inhibition of human C5 protein expression or expression of an mRNA encoding human C5 protein) are well known in the art of molecular biology and include, e.g., Northern blot and RT-PCR (or quantitative RT-PCR) techniques for mRNA and for protein detection, Western blot, dot blot, or ELISA techniques. (See, e.g., Sambrook et al. (1989) "Molecular Cloning: A Laboratory Manual, 2nd Edition," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.)

Methods for determining whether a candidate compound inhibits the cleavage of human C5 into forms C5a and C5b are known in the art and described in, e.g., Moongkarndi et al. (1982) *Immunobiol.* 162:397; Moongkarndi et al. (1983) *Immunobiol.* 165:323; Isenman et al. (1980) *J Immunol.* 124(1):326-31; Thomas et al. (1996) *Mol. Immunol.* 33(17-18):1389-401; and Evans et al. (1995) *Mol. Immunol.* 32(16):1183-95.

Inhibition of human complement component C5 can also reduce the cell-lysing ability of complement in a subject's body fluids. Such reductions of the cell-lysing ability of complement present can be measured by methods well known in the art such as, for example, by a conventional hemolytic assay such as the hemolysis assay described by Kabat and Mayer (eds), "Experimental Immunochemistry, 2nd Edition," 135-240, Springfield, IL, CC Thomas (1961), pages 135-139, or a conventional variation of that assay such as the chicken erythrocyte hemolysis method as described in, e.g., Hillmen et al. (2004) *N Engl J Med* 350(6):552.

Pharmaceutical Compositions and Formulations. The compositions containing a complement inhibitor (e.g., an inhibitor of human complement component C5 such as an anti-C5 antibody or antigen-binding fragment thereof) can be formulated as a pharmaceutical composition, e.g., for administration to a subject to treat aHUS, CAPS, Degos disease, or TMA. The pharmaceutical compositions will generally include a pharmaceutically acceptable carrier. As used herein, a "pharmaceutically acceptable carrier" refers to, and includes, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The compositions can include a pharmaceutically acceptable salt, e.g., an acid addition salt or a base addition salt (see e.g., Berge et al. (1977) *J. Pharm. Sci.* 66:1-19).

The compositions can be formulated according to standard methods. Pharmaceutical formulation is a well-established art, and is further described in, e.g., Gennaro (2000) "Remington: The Science and Practice of Pharmacy," 20th Edition, Lippincott, Williams & Wilkins (ISBN: 0683306472); Ansel et al. (1999) "Pharmaceutical Dosage Forms and Drug Delivery Systems," 7th Edition, Lippincott Williams & Wilkins Publishers (ISBN: 0683305727); and Kibbe (2000) "Handbook of Pharmaceutical Excipients American Pharmaceutical Association," 3rd Edition (ISBN: 091733096X). In some embodiments, a composition can be formulated, for example, as a buffered solution at a suitable concentration and suitable for storage at 2-8°C. In some embodiments, a composition can be formulated for storage at a temperature below 0°C (e.g., -20°C or -80°C).

The pharmaceutical compositions can be in a variety of forms. These forms include, e.g., liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends, in part, on the intended mode of administration and therapeutic application. For example, compositions containing an anti-C5 antibody intended for systemic or local delivery can be in the form of injectable or infusible solutions. Accordingly, the compositions can be formulated for administration by a parenteral mode (e.g., intravenous, subcutaneous, intraperitoneal, or intramuscular injection). "Parenteral administration," "administered parenterally," and other grammatically equivalent phrases, as used herein, refer to modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, intravenous, intranasal, intraocular, pulmonary, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intrapulmonary, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intracerebral, intracranial, intracarotid and intrasternal injection and infusion (see below).

The compositions can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable for stable storage at high concentration. Sterile injectable solutions can be prepared by incorporating an antibody described herein in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating an inhibitor of human complement component C5 (e.g., an anti-C5 antibody) described herein into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods for preparation include vacuum drying and freeze-drying that yield a powder of the antibody described herein plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition a reagent that delays absorption, for example, monostearate salts and gelatin.

In certain embodiments, the C5 inhibitor (e.g., an anti-C5 antibody or antigen-binding fragment thereof) can be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are known in the art. (See, e.g., J.R. Robinson (1978) "Sustained and Controlled Release Drug Delivery Systems," Marcel Dekker, Inc., New York.)

In some embodiments, an antibody described herein can be formulated in a composition suitable for intrapulmonary administration (e.g., for administration via nebulizer) to a mammal such as a human. Methods for preparing such compositions are well known in the art and described in, e.g., U.S. Patent Application Publication No. 20080202513; U.S. Patent Nos. 7,112,341 and 6,019,968; and PCT Publication Nos. WO 00/061178 and WO 06/122257, the disclosures of each of which are incorporated herein by reference in their entirety. Dry powder inhaler formulations and suitable systems for administration of the formulations are described in, e.g., U.S. Patent Application Publication No. 20070235029, PCT Publication No. WO 00/69887; and U.S. Patent No. 5,997,848.

In some embodiments, an inhibitor of human C5 (e.g., an anti-C5 antibody or antigen-binding fragment thereof) described herein can be modified, e.g., with a

moiety that improves its stabilization and/or retention in circulation, e.g., in blood, serum, or other tissues. The stabilization moiety can improve the stability, or retention of, the antibody by at least 1.5 (e.g., at least 2, 5, 10, 15, 20, 25, 30, 40, or 50 or more) fold.

The nucleic acid inhibitors of human complement component C5 described herein (e.g., an anti-sense nucleic acid or siRNA) can be incorporated into a gene construct to be used as a part of a gene therapy protocol to deliver nucleic acids that can be used to express and produce agents within cells. Expression constructs of such components may be administered in any biologically effective carrier, e.g. any formulation or composition capable of effectively delivering the component gene to cells *in vivo*. Approaches include insertion of the subject gene in viral vectors including recombinant retroviruses, adenovirus, adeno-associated virus, lentivirus, and herpes simplex virus-1 (HSV-1), or recombinant bacterial or eukaryotic plasmids. Viral vectors can transfect cells directly; plasmid DNA can be delivered with the help of, for example, cationic liposomes (lipofectin) or derivatized (e.g., antibody conjugated), polylysine conjugates, gramicidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or $CaPO_4$ precipitation carried out *in vivo*. (See also, "*Ex vivo* Approaches," below.) Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are known to those skilled in the art (see, e.g., Eglitis et al. (1985) *Science* 230:1395-1398; Danos and Mulligan (1988) *Proc. Natl. Acad. Sci. USA* 85:6460-6464; Wilson et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:3014-3018; Armentano et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6141-6145; Huber et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8039-8043; Ferry et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8377-8381; Chowdhury et al. (1991) *Science* 254:1802-1805; van Beusechem et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:7640-7644; Kay et al. (1992) *Human Gene Therapy* 3:641-647; Dai et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:10892-10895; Hwu et al. (1993) *J. Immunol.* 150:4104-4115; U.S. Patent Nos. 4,868,116 and 4,980,286; PCT Publication Nos. WO89/07136, WO89/02468, WO89/05345, and WO92/07573). Another viral gene delivery system utilizes adenovirus-derived vectors (see, e.g., Berkner et al. (1988) *BioTechniques* 6:616; Rosenfeld et al. (1991) *Science* 252:431-434; and Rosenfeld et al. (1992) *Cell* 68:143-155). Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3, Ad7, etc.) are known to those

skilled in the art. Yet another viral vector system useful for delivery of the subject gene is the adeno-associated virus (AAV). See, e.g., Flotte et al. (1992) *Am. J. Respir. Cell. Mol. Biol.* 7:349-356; Samulski et al. (1989) *J. Virol.* 63:3822-3828; and McLaughlin et al. (1989) *J. Virol* 62: 1963-1973.

In some embodiments, more than one (e.g., two, three, four, five, six, seven, eight, nine, or 10 or more) inhibitor(s) (e.g., one or more inhibitors of human C5) can be co-formulated. For example, a C5-specific siRNA and an anti-C5 antibody can be formulated together.

In some embodiments, an inhibitor of human complement (e.g., an inhibitor human C5 such as an anti-C5 antibody or antigen-binding fragment thereof) described herein can be formulated with one or more additional active agents useful for treating a complement-associated disorder (e.g., any of the complement-associated disorders described herein such as APS, CAPS, aHUS, Degos disease, HELLP syndrome) or ameliorating a symptom thereof. For example, an anti-C5 antibody can be formulated with an antihypertensive, an anticoagulant, and/or a steroid (e.g., a corticosteroid). Examples of anticoagulants include, e.g., warfarin (Coumadin), aspirin, heparin, phenindione, fondaparinux, idraparinux, and thrombin inhibitors (e.g., argatroban, lepirudin, bivalirudin, or dabigatran). An inhibitor of human C5 (e.g., an anti-C5 antibody, an anti-C5a antibody, or an anti-C5b antibody) can also be formulated with a fibrinolytic agent (e.g., ancrod, ε-aminocaproic acid, antiplasmin-a_1, prostacyclin, and defibrotide), cyclophosphamide, or an anti-cytokine agent for the treatment of CAPS. Anti-cytokine agents include, e.g., antibodies or soluble receptors that bind to and modulate the activity of cytokine (e.g., a pro-inflammatory cytokine such as TNF). Examples of anti-cytokine agents include, e.g., a TNF inhibitor such as a soluble TNF receptor (e.g., etanercept; Enbrel®) or an anti-TNF antibody (e.g., infliximab; Remicade®). In some embodiments, the inhibitor can be formulated with, or for use with, an anti-CD20 agent such as rituximab (Rituxan™; Biogen Idec, Cambridge, MA). In some embodiments, the inhibitor of human C5 can be formulated for administration to a subject along with intravenous immunoglobulin therapy (IVIG) or with plasma exchange.

When the inhibitor of human C5 is to be used in combination with a second active agent, or when two or more inhibitors of human C5 are to be used (e.g., an anti-C5a antibody and an anti-C5b antibody), the agents can be formulated separately or

together. For example, the respective pharmaceutical compositions can be mixed, e.g., just prior to administration, and administered together or can be administered separately, e.g., at the same or different times (see below).

As described above, a composition can be formulated such that it includes a therapeutically effective amount of an inhibitor of human C5 (e.g., an anti-C5 antibody or antigen-binding fragment thereof) or the composition can be formulated to include a sub-therapeutic amount of the inhibitor and a sub-therapeutic amount of one or more additional active agents such that the components in total are therapeutically effective for treating a complement-associated disorder such as any of those described herein. In some embodiments, a composition can be formulated to include two or more inhibitors of human C5, each at sub-therapeutic doses, such that the inhibitors in total are at a concentration that is therapeutically effective for treating a complement-associated disorder such as, e.g., aHUS, CAPS, Degos disease, or HELLP syndrome. Methods for determining a therapeutically effective dose (e.g., a therapeutically effective dose of an anti-C5 antibody) are known in the art and described herein.

Methods for Producing an Antibody

Suitable methods for producing an antibody (e.g., an anti-C5 antibody, an anti-C5a antibody, and/or an anti-C5b antibody), or antigen-binding fragments thereof, in accordance with the disclosure are known in the art (see, e.g., U.S. Patent No. 6,355,245) and described herein. For example, monoclonal anti-C5 antibodies may be generated using complement component C5-expressing cells, a C5 polypeptide, or an antigenic fragment of C5 polypeptide (e.g., C5a or C5b), as an immunogen, thus raising an immune response in animals from which antibody-producing cells and in turn monoclonal antibodies may be isolated. The sequence of such antibodies may be determined and the antibodies or variants thereof produced by recombinant techniques. Recombinant techniques may be used to produce chimeric, CDR-grafted, humanized and fully human antibodies based on the sequence of the monoclonal antibodies as well as polypeptides capable of binding to human complement component C5.

Moreover, antibodies derived from recombinant libraries ("phage antibodies") may be selected using, e.g., C5-expressing cells, or polypeptides derived therefrom, as

bait to isolate the antibodies or polypeptides on the basis of target specificity. The production and isolation of non-human and chimeric anti-C5 antibodies are well within the purview of the skilled artisan.

Recombinant DNA technology can be used to modify one or more characteristics of the antibodies produced in non-human cells. Thus, chimeric antibodies can be constructed in order to decrease the immunogenicity thereof in diagnostic or therapeutic applications. Moreover, immunogenicity can be minimized by humanizing the antibodies by CDR grafting and, optionally, framework modification. See, U.S. Patent Nos. 5,225,539 and 7,393,648, the contents of each of which are incorporated herein by reference.

Antibodies can be obtained from animal serum or, in the case of monoclonal antibodies or fragments thereof, produced in cell culture. Recombinant DNA technology can be used to produce the antibodies according to established procedure, including procedures in bacterial or preferably mammalian cell culture. The selected cell culture system preferably secretes the antibody product.

In another embodiment, a process for the production of an antibody disclosed herein includes culturing a host, e.g., *E. coli* or a mammalian cell, which has been transformed with a hybrid vector. The vector includes one or more expression cassettes containing a promoter operably linked to a first DNA sequence encoding a signal peptide linked in the proper reading frame to a second DNA sequence encoding the antibody protein. The antibody protein is then collected and isolated. Optionally, the expression cassette may include a promoter operably linked to polycistronic (e.g., bicistronic) DNA sequences encoding antibody proteins each individually operably linked to a signal peptide in the proper reading frame.

Multiplication of hybridoma cells or mammalian host cells *in vitro* is carried out in suitable culture media, which include the customary standard culture media (such as, for example Dulbecco's Modified Eagle Medium (DMEM) or RPMI 1640 medium), optionally replenished by a mammalian serum (e.g. fetal calf serum), or trace elements and growth sustaining supplements (e.g. feeder cells such as normal mouse peritoneal exudate cells, spleen cells, bone marrow macrophages, 2-aminoethanol, insulin, transferrin, low density lipoprotein, oleic acid, or the like). Multiplication of host cells which are bacterial cells or yeast cells is likewise carried out in suitable culture media known in the art. For example, for bacteria suitable

culture media include medium LE, NZCYM, NZYM, NZM, Terrific Broth, SOB, SOC, 2 xYT, or M9 Minimal Medium. For yeast, suitable culture media include medium YPD, YEPD, Minimal Medium, or Complete Minimal Dropout Medium.

In vitro production provides relatively pure antibody preparations and allows scale-up production to give large amounts of the desired antibodies. Techniques for bacterial cell, yeast, plant, or mammalian cell cultivation are known in the art and include homogeneous suspension culture (e.g. in an airlift reactor or in a continuous stirrer reactor), and immobilized or entrapped cell culture (e.g. in hollow fibers, microcapsules, on agarose microbeads or ceramic cartridges).

Large quantities of the desired antibodies can also be obtained by multiplying mammalian cells *in vivo*. For this purpose, hybridoma cells producing the desired antibodies are injected into histocompatible mammals to cause growth of antibody-producing tumors. Optionally, the animals are primed with a hydrocarbon, especially mineral oils such as pristane (tetramethyl-pentadecane), prior to the injection. After one to three weeks, the antibodies are isolated from the body fluids of those mammals. For example, hybridoma cells obtained by fusion of suitable myeloma cells with antibody-producing spleen cells from Balb/c mice, or transfected cells derived from hybridoma cell line Sp2/0 that produce the desired antibodies are injected intraperitoneally into Balb/c mice optionally pre-treated with pristane. After one to two weeks, ascitic fluid is taken from the animals.

The foregoing, and other, techniques are discussed in, for example, Kohler and Milstein, (1975) *Nature* 256:495-497; U.S. Patent No. 4,376,110; Harlow and Lane, Antibodies: a Laboratory Manual, (1988) Cold Spring Harbor, the disclosures of which are all incorporated herein by reference. Techniques for the preparation of recombinant antibody molecules are described in the above references and also in, e.g.:WO97/08320; U.S. Patent No. 5,427,908; U.S. Patent No. 5,508,717; Smith (1985) *Science* 225:1315-1317; Parmley and Smith (1988) *Gene* 73:305-318; De La Cruz et al. (1988) *Journal of Biological Chemistry* 263:4318-4322; U.S. Patent No. 5,403,484; U.S. Patent No. 5,223,409; WO88/06630; WO92/15679; U.S. Patent No. 5,780,279; U.S. Patent No. 5,571,698; U.S. Patent No. 6,040,136; Davis et al. (1999) *Cancer Metastasis Rev.* 18(4):421-5; and Taylor et al. (1992) *Nucleic Acids Research* 20: 6287-6295; Tomizuka et al. (2000) *Proc. Natl. Acad. Sci. USA* 97(2): 722-727, the contents of each of which are incorporated herein by reference in their entirety.

The cell culture supernatants are screened for the desired antibodies, preferentially by immunofluorescent staining of complement component C5-expressing cells, by immunoblotting, by an enzyme immunoassay, e.g. a sandwich assay or a dot-assay, or a radioimmunoassay.

For isolation of the antibodies, the immunoglobulins in the culture supernatants or in the ascitic fluid may be concentrated, e.g., by precipitation with ammonium sulfate, dialysis against hygroscopic material such as polyethylene glycol, filtration through selective membranes, or the like. If necessary and/or desired, the antibodies are purified by the customary chromatography methods, for example gel filtration, ion-exchange chromatography, chromatography over DEAE-cellulose and/or (immuno-) affinity chromatography, e.g. affinity chromatography with one or more surface polypeptides derived from a complement component C5-expressing cell line, or with Protein-A or -G.

Another embodiment provides a process for the preparation of a bacterial cell line secreting antibodies directed against a C5 protein in a suitable mammal. For example a rabbit is immunized with pooled samples from C5-expressing tissue or cells or C5 polypeptide or fragments thereof. A phage display library produced from the immunized rabbit is constructed and panned for the desired antibodies in accordance with methods well known in the art (such as, e.g., the methods disclosed in the various references incorporated herein by reference).

Hybridoma cells secreting the monoclonal antibodies are also disclosed. The preferred hybridoma cells are genetically stable, secrete monoclonal antibodies described herein of the desired specificity, and can be expanded from deep-frozen cultures by thawing and propagation *in vitro* or as ascites *in vivo*.

In another embodiment, a process is provided for the preparation of a hybridoma cell line secreting monoclonal antibodies against a complement component C5 protein. In that process, a suitable mammal, for example a Balb/c mouse, is immunized with one or more polypeptides or antigenic fragments of C5 or with one or more polypeptides or antigenic fragments derived from a C5-expressing cell, the C5-expressing cell itself, or an antigenic carrier containing a purified polypeptide as described. Antibody-producing cells of the immunized mammal are grown briefly in culture or fused with cells of a suitable myeloma cell line. The hybrid cells obtained in the fusion are cloned, and cell clones secreting the desired

antibodies are selected. For example, spleen cells of Balb/c mice immunized with a C5-expressing Chronic Lymphocytic Leukemia (CLL) cell line are fused with cells of the myeloma cell line PAI or the myeloma cell line Sp2/0-Ag 14. The obtained hybrid cells are then screened for secretion of the desired antibodies and positive hybridoma cells are cloned.

Methods for preparing a hybridoma cell line include immunizing Balb/c mice by injecting subcutaneously and/or intraperitoneally an immunogenic composition containing human C5 protein (or an immunogenic fragment thereof) several times, e.g., four to six times, over several months, e.g., between two and four months. Spleen cells from the immunized mice are taken two to four days after the last injection and fused with cells of the myeloma cell line PAI in the presence of a fusion promoter, preferably polyethylene glycol. Preferably, the myeloma cells are fused with a three- to twenty-fold excess of spleen cells from the immunized mice in a solution containing about 30% to about 50% polyethylene glycol of a molecular weight around 4000. After the fusion, the cells are expanded in suitable culture media as described *supra*, supplemented with a selection medium, for example HAT medium, at regular intervals in order to prevent normal myeloma cells from overgrowing the desired hybridoma cells.

The antibodies and fragments thereof can be "chimeric." Chimeric antibodies and antigen-binding fragments thereof comprise portions from two or more different species (e.g., mouse and human). Chimeric antibodies can be produced with mouse variable regions of desired specificity spliced onto human constant domain gene segments (for example, U.S. Patent No. 4,816,567). In this manner, non-human antibodies can be modified to make them more suitable for human clinical application (e.g., methods for treating or preventing a complement associated disorder in a human subject).

The monoclonal antibodies of the present disclosure include "humanized" forms of the non-human (e.g., mouse) antibodies. Humanized or CDR-grafted mAbs are particularly useful as therapeutic agents for humans because they are not cleared from the circulation as rapidly as mouse antibodies and do not typically provoke an adverse immune reaction. Methods of preparing humanized antibodies are generally well known in the art. For example, humanization can be essentially performed following the method of Winter and co-workers (see, e.g., Jones et al. (1986) *Nature*

321:522-525; Riechmann et al. (1988) *Nature* 332:323-327; and Verhoeyen et al. (1988) *Science* 239:1534-1536), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Also see, e.g., Staelens et al. (2006) *Mol Immunol* 43:1243-1257. In some embodiments, humanized forms of non-human (e.g., mouse) antibodies are human antibodies (recipient antibody) in which hypervariable (CDR) region residues of the recipient antibody are replaced by hypervariable region residues from a non-human species (donor antibody) such as a mouse, rat, rabbit, or non-human primate having the desired specificity, affinity, and binding capacity. In some instances, framework region residues of the human immunoglobulin are also replaced by corresponding non-human residues (so called "back mutations"). In addition, phage display libraries can be used to vary amino acids at chosen positions within the antibody sequence. The properties of a humanized antibody are also affected by the choice of the human framework. Furthermore, humanized and chimerized antibodies can be modified to comprise residues that are not found in the recipient antibody or in the donor antibody in order to further improve antibody properties, such as, for example, affinity or effector function.

Fully human antibodies are also provided in the disclosure. The term "human antibody" includes antibodies having variable and constant regions (if present) derived from human germline immunoglobulin sequences. Human antibodies can include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody" does not include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences (i.e., humanized antibodies). Fully human or human antibodies may be derived from transgenic mice carrying human antibody genes (carrying the variable (V), diversity (D), joining (J), and constant (C) exons) or from human cells. For example, it is now 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. (See, e.g., Jakobovits et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:2551; Jakobovits et al. (1993) *Nature* 362:255-258; Bruggemann et al. (1993) *Year in Immunol.* 7:33; and Duchosal et al. (1992)

Nature 355:258.) Transgenic mice strains can be engineered to contain gene sequences from unrearranged human immunoglobulin genes. The human sequences may code for both the heavy and light chains of human antibodies and would function correctly in the mice, undergoing rearrangement to provide a wide antibody repertoire similar to that in humans. The transgenic mice can be immunized with the target protein (e.g., a complement component C5 protein, fragments thereof, or cells expressing C5 protein) to create a diverse array of specific antibodies and their encoding RNA. Nucleic acids encoding the antibody chain components of such antibodies may then be cloned from the animal into a display vector. Typically, separate populations of nucleic acids encoding heavy and light chain sequences are cloned, and the separate populations then recombined on insertion into the vector, such that any given copy of the vector receives a random combination of a heavy and a light chain. The vector is designed to express antibody chains so that they can be assembled and displayed on the outer surface of a display package containing the vector. For example, antibody chains can be expressed as fusion proteins with a phage coat protein from the outer surface of the phage. Thereafter, display packages can be screened for display of antibodies binding to a target.

In addition, human antibodies can be derived from phage-display libraries (Hoogenboom et al. (1991) *J. Mol. Biol.* 227:381; Marks et al. (1991) *J. Mol. Biol.*, 222:581-597; and Vaughan et al. (1996) *Nature Biotech* 14:309 (1996)). Synthetic phage libraries can be created which use randomized combinations of synthetic human antibody V-regions. By selection on antigen fully human antibodies can be made in which the V-regions are very human-like in nature. See, e.g., U.S. Patent Nos. 6,794,132, 6,680,209, 4,634,666, and Ostberg et al. (1983), *Hybridoma* 2:361-367, the contents of each of which are incorporated herein by reference in their entirety.

For the generation of human antibodies, also see Mendez et al. (1998) *Nature Genetics* 15:146-156, Green and Jakobovits (1998) *J. Exp. Med.* 188:483-495, the disclosures of which are hereby incorporated by reference in their entirety. Human antibodies are further discussed and delineated in U.S. Patent Nos.: 5,939,598; 6,673,986; 6,114,598; 6,075,181; 6,162,963; 6,150,584; 6,713,610; and 6,657,103 as well as U.S. Patent Application Publication Nos. 2003-0229905 A1, 2004-0010810 A1, US 2004-0093622 A1, 2006-0040363 A1, 2005-0054055 A1, 2005-0076395 A1,

2005-0287630 A1. See also International Publication Nos. WO 94/02602, WO 96/34096, and WO 98/24893, and European Patent No. EP 0 463 151 B1. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

In an alternative approach, others, including GenPharm International, Inc., have utilized a "minilocus" approach. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant region, and a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in, e.g., U.S. Patent Nos.: 5,545,807; 5,545,806; 5,625,825; 5,625,126; 5,633,425; 5,661,016; 5,770,429; 5,789,650; and 5,814,318; 5,591,669; 5,612,205; 5,721,367; 5,789,215; 5,643,763; 5,569,825; 5,877,397; 6,300,129; 5,874,299; 6,255,458; and 7,041,871, the disclosures of which are hereby incorporated by reference. See also European Patent No. 0 546 073 B1, International Patent Publication Nos. WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884, the disclosures of each of which are hereby incorporated by reference in their entirety. See further Taylor et al. (1992) *Nucleic Acids Res.* 20: 6287; Chen et al. (1993) *Int. Immunol.* 5: 647; Tuaillon et al. (1993) *Proc. Natl. Acad. Sci. USA* 90: 3720-4; Choi et al. (1993) *Nature Genetics* 4: 117; Lonberg et al. (1994) *Nature* 368: 856-859; Taylor et al. (1994) *International Immunology* 6: 579-591; Tuaillon et al. (1995) *J. Immunol.* 154: 6453-65; Fishwild et al. (1996) *Nature Biotechnology* 14: 845; and Tuaillon et al. (2000) *Eur. J. Immunol.* 10: 2998-3005, the disclosures of each of which are hereby incorporated by reference in their entirety.

In certain embodiments, de-immunized anti-C5 antibodies or antigen-binding fragments thereof are provided. De-immunized antibodies or antigen-binding fragments thereof are antibodies that have been modified so as to render the antibody or antigen-binding fragment thereof non-immunogenic, or less immunogenic, to a given species (e.g., to a human). De-immunization can be achieved by modifying the antibody or antigen-binding fragment thereof utilizing any of a variety of techniques known to those skilled in the art (see, e.g., PCT Publication Nos. WO 04/108158 and WO 00/34317). For example, an antibody or antigen-binding fragment thereof may

be de-immunized by identifying potential T cell epitopes and/or B cell epitopes within the amino acid sequence of the antibody or antigen-binding fragment thereof and removing one or more of the potential T cell epitopes and/or B cell epitopes from the antibody or antigen-binding fragment thereof, for example, using recombinant techniques. The modified antibody or antigen-binding fragment thereof may then optionally be produced and tested to identify antibodies or antigen-binding fragments thereof that have retained one or more desired biological activities, such as, for example, binding affinity, but have reduced immunogenicity. Methods for identifying potential T cell epitopes and/or B cell epitopes may be carried out using techniques known in the art, such as, for example, computational methods (see e.g., PCT Publication No. WO 02/069232), *in vitro* or *in silico* techniques, and biological assays or physical methods (such as, for example, determination of the binding of peptides to MHC molecules, determination of the binding of peptide:MHC complexes to the T cell receptors from the species to receive the antibody or antigen-binding fragment thereof, testing of the protein or peptide parts thereof using transgenic animals with the MHC molecules of the species to receive the antibody or antigen-binding fragment thereof, or testing with transgenic animals reconstituted with immune system cells from the species to receive the antibody or antigen-binding fragment thereof, etc.). In various embodiments, the de-immunized anti-C5 antibodies described herein include de-immunized antigen-binding fragments, Fab, Fv, scFv, Fab' and $F(ab')_2$, monoclonal antibodies, murine antibodies, engineered antibodies (such as, for example, chimeric, single chain, CDR-grafted, humanized, fully human antibodies, and artificially selected antibodies), synthetic antibodies and semi-synthetic antibodies.

In some embodiments, a recombinant DNA comprising an insert coding for a heavy chain variable domain and/or for a light chain variable domain of an anti-C5 antibody or a C5 protein-expressing cell line is produced. The term DNA includes coding single stranded DNAs, double stranded DNAs consisting of said coding DNAs and of complementary DNAs thereto, or these complementary (single stranded) DNAs themselves.

Furthermore, a DNA encoding a heavy chain variable domain and/or a light chain variable domain of anti-C5 antibodies can be enzymatically or chemically synthesized to contain the authentic DNA sequence coding for a heavy chain variable

domain and/or for the light chain variable domain, or a mutant thereof. A mutant of the authentic DNA is a DNA encoding a heavy chain variable domain and/or a light chain variable domain of the above-mentioned antibodies in which one or more amino acids are deleted, inserted, or exchanged with one or more other amino acids. Preferably said modification(s) are outside the CDRs of the heavy chain variable domain and/or of the light chain variable domain of the antibody in humanization and expression optimization applications. The term mutant DNA also embraces silent mutants wherein one or more nucleotides are replaced by other nucleotides with the new codons coding for the same amino acid(s). The term mutant sequence also includes a degenerate sequence. Degenerate sequences are degenerate within the meaning of the genetic code in that an unlimited number of nucleotides are replaced by other nucleotides without resulting in a change of the amino acid sequence originally encoded. Such degenerate sequences may be useful due to their different restriction sites and/or frequency of particular codons which are preferred by the specific host, particularly *E. coli*, to obtain an optimal expression of the heavy chain murine variable domain and/or a light chain murine variable domain.

The term mutant is intended to include a DNA mutant obtained by *in vitro* mutagenesis of the authentic DNA according to methods known in the art.

For the assembly of complete tetrameric immunoglobulin molecules and the expression of chimeric antibodies, the recombinant DNA inserts coding for heavy and light chain variable domains are fused with the corresponding DNAs coding for heavy and light chain constant domains, then transferred into appropriate host cells, for example after incorporation into hybrid vectors.

Recombinant DNAs including an insert coding for a heavy chain murine variable domain of an anti-C5 antibody or a C5-expressing cell line fused to a human constant domain IgG, for example γ1, γ2, γ3 or γ4, in particular embodiments γ1 or γ4, may be used. Recombinant DNAs including an insert coding for a light chain murine variable domain of an antibody fused to a human constant domain κ or λ, preferably κ, are also provided.

Another embodiment pertains to recombinant DNAs coding for a recombinant polypeptide wherein the heavy chain variable domain and the light chain variable domain are linked by way of a spacer group, optionally comprising a signal sequence facilitating the processing of the antibody in the host cell and/or a DNA sequence

encoding a peptide facilitating the purification of the antibody and/or a cleavage site and/or a peptide spacer and/or an agent.

Accordingly, the monoclonal antibodies or antigen-binding fragments of the disclosure can be naked antibodies or antigen-binding fragments that are not conjugated to other agents, for example, a therapeutic agent or detectable label. Alternatively, the monoclonal antibody or antigen-binding fragment can be conjugated to an agent such as, for example, a cytotoxic agent, a small molecule, a hormone, an enzyme, a growth factor, a cytokine, a ribozyme, a peptidomimetic, a chemical, a prodrug, a nucleic acid molecule including coding sequences (such as antisense, RNAi, gene-targeting constructs, etc.), or a detectable label (e.g., an NMR or X-ray contrasting agent, fluorescent molecule, etc.). In certain embodiments, an anti-C5 antibody or antigen-binding fragment (e.g., Fab, Fv, single-chain scFv, Fab', and $F(ab')_2$) is linked to a molecule that increases the half-life of the antibody or antigen-binding fragment (see above).

Several possible vector systems are available for the expression of cloned heavy chain and light chain genes in mammalian cells. One class of vectors relies upon the integration of the desired gene sequences into the host cell genome. Cells which have stably integrated DNA can be selected by simultaneously introducing drug resistance genes such as *E. coli* gpt (Mulligan and Berg (1981) *Proc. Natl. Acad. Sci. USA*, 78:2072) or Tn5 neo (Southern and Berg (1982) *Mol. Appl. Genet.* 1:327). The selectable marker gene can be either linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection (Wigler et al. (1979) *Cell* 16:77). A second class of vectors utilizes DNA elements which confer autonomously replicating capabilities to an extrachromosomal plasmid. These vectors can be derived from animal viruses, such as bovine papillomavirus (Sarver et al. (1982) *Proc. Natl. Acad. Sci. USA*, 79:7147), polyoma virus (Deans et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:1292), or SV40 virus (Lusky and Botchan (1981) *Nature* 293:79).

Since an immunoglobulin cDNA is comprised only of sequences representing the mature mRNA encoding an antibody protein, additional gene expression elements regulating transcription of the gene and processing of the RNA are required for the synthesis of immunoglobulin mRNA. These elements may include splice signals, transcription promoters, including inducible promoters, enhancers, and termination

signals. cDNA expression vectors incorporating such elements include those described by Okayama and Berg (1983) *Mol. Cell Biol.* 3:280; Cepko et al. (1984) *Cell* 37:1053; and Kaufman (1985) *Proc. Natl. Acad. Sci. USA* 82:689.

As is evident from the disclosure, antibodies that binds to human complement components (e.g., antibodies that bind to C5, C5b, or C5a) can be used in therapies (e.g., therapies for a complement-associated disorder), including combination therapies, as well as in the monitoring of disease progression.

In the therapeutic embodiments of the present disclosure, bispecific antibodies are contemplated. Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the human complement component C5 antigen the other one is for any other antigen.

Methods for making bispecific antibodies are within the purview of those skilled in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello (1983) *Nature* 305:537-539). Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, including at least part of the hinge, C_H2, and C_H3 regions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of illustrative currently known methods for generating bispecific antibodies see, e.g., Suresh et al. (1986) *Methods in Enzymology* 121:210; PCT Publication No. WO 96/27011; Brennan et al. (1985) *Science* 229:81; Shalaby et al., *J. Exp. Med.* (1992) 175:217-225; Kostelny et al. (1992) *J. Immunol.* 148(5):1547-1553; Hollinger et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Gruber et al. (1994) *J. Immunol.* 152:5368; and Tutt et al. (1991) J. Immunol. 147:60. Bispecific antibodies also include cross-linked or heteroconjugate antibodies. Heteroconjugate antibodies may be made using any convenient cross-linking methods. 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.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. (See, e.g., Kostelny et al. (1992) *J. Immunol.* 148(5):1547-1553). The leucine zipper peptides from the Fos and Jun proteins may be linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers may be reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448 has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (scFv) dimers has also been reported. (See, e.g., Gruber et al. (1994) *J. Immunol.* 152:5368.) Alternatively, the antibodies can be "linear antibodies" as described in, e.g., Zapata et al. (1995) *Protein Eng.* 8(10):1057-1062. Briefly, these antibodies comprise a pair of tandem Fd segments (V_H-C_H1-V_H-C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

The disclosure also embraces variant forms of bispecific antibodies such as the tetravalent dual variable domain immunoglobulin (DVD-Ig) molecules described in Wu et al. (2007) *Nat Biotechnol* 25(11):1290-1297. The DVD-Ig molecules are designed such that two different light chain variable domains (VL) from two different parent antibodies are linked in tandem directly or via a short linker by recombinant DNA techniques, followed by the light chain constant domain. Methods for generating DVD-Ig molecules from two parent antibodies are further described in, e.g., PCT Publication Nos. WO 08/024188 and WO 07/024715, the disclosures of each of which are incorporated herein by reference in their entirety.

Methods for Treatment

The above-described compositions (e.g., any of the C5 inhibitors described herein or pharmaceutical compositions thereof) are useful in, *inter alia*, methods for treating or preventing a variety of complement-associated disorders (e.g., AP-associated disorders or CP-associated disorders) in a subject. The compositions can be administered to a subject, e.g., a human subject, using a variety of methods that depend, in part, on the route of administration. The route can be, e.g., intravenous injection or infusion (IV), subcutaneous injection (SC), intraperitoneal (IP), intrapulmonary, intraocular, or intramuscular injection. Certain inhibitors, e.g., small molecules, can be orally administered to a subject.

Administration can be achieved by, e.g., local infusion, injection, or by means of an implant. The implant can be of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. The implant can be configured for sustained or periodic release of the composition to the subject. (See, e.g., U.S. Patent Application Publication No. 20080241223; U.S. Patent Nos. 5,501,856; 4,863,457; and 3,710,795; EP488401; and EP 430539, the disclosures of each of which are incorporated herein by reference in their entirety.) The composition can be delivered to the subject by way of an implantable device based on, e.g., diffusive, erodible, or convective systems, e.g., osmotic pumps, biodegradable implants, electrodiffusion systems, electroosmosis systems, vapor pressure pumps, electrolytic pumps, effervescent pumps, piezoelectric pumps, erosion-based systems, or electromechanical systems.

A suitable dose of a complement inhibitor (e.g., a C5 inhibitor such as an anti-C5 antibody) described herein, which dose is capable of treating or preventing a complement-associated disorder in a subject, can depend on a variety of factors including, e.g., the age, sex, and weight of a subject to be treated and the particular inhibitor compound used. For example, a different dose of an anti-C5 antibody may be required to treat a subject with RA as compared to the dose of a C5-specific siRNA molecule that is required to treat the same subject. Other factors affecting the dose administered to the subject include, e.g., the type or severity of the complement-associated disorder. For example, a subject having RA may require administration of a different dosage of an anti-C5 antibody than a subject with AMD. Other factors can include, e.g., other medical disorders concurrently or previously affecting the subject,

the general health of the subject, the genetic disposition of the subject, diet, time of administration, rate of excretion, drug combination, and any other additional therapeutics that are administered to the subject. It should also be understood that a specific dosage and treatment regimen for any particular subject will depend upon the judgment of the treating medical practitioner (e.g., doctor or nurse).

An antibody described herein can be administered as a fixed dose, or in a milligram per kilogram (mg/kg) dose. In some embodiments, the dose can also be chosen to reduce or avoid production of antibodies or other host immune responses against one or more of the active antibodies in the composition. While in no way intended to be limiting, exemplary dosages of an antibody include, e.g., 1-100 μg/kg, 0.5-50 μg/kg, 0.1-100 μg/kg, 0.5-25 μg/kg, 1-20 μg/kg, and 1-10 μg/kg, 1-100 mg/kg, 0.5-50 mg/kg, 0.1-100 mg/kg, 0.5-25 mg/kg, 1-20 mg/kg, and 1-10 mg/kg. Exemplary dosages of an antibody described herein include, without limitation, 0.1 μg/kg, 0.5 μg/kg, 1.0 μg/kg, 2.0 μg/kg, 4 μg/kg, and 8 μg/kg, 0.1 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 4 mg/kg, and 8 mg/kg. Further exemplary dosage amounts and schedules are provided herein (see, e.g., Tables 1 and 2).

A pharmaceutical composition can include a therapeutically effective amount of a complement inhibitor (e.g., a C5 inhibitor such as an anti-C5 antibody) described herein. Such effective amounts can be readily determined by one of ordinary skill in the art based, in part, on the effect of the administered antibody, or the combinatorial effect of the antibody and one or more additional active agents, if more than one agent is used. A therapeutically effective amount of an antibody described herein can also vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody (and one or more additional active agents) to elicit a desired response in the individual, e.g., amelioration of at least one condition parameter, e.g., amelioration of at least one symptom of the complement-associated disorder. For example, a therapeutically effective amount of an antibody that binds to C5a and C5b can inhibit (lessen the severity of or eliminate the occurrence of) and/or prevent a particular disorder, and/or any one of the symptoms of the particular disorder known in the art or described herein. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects.

Suitable human doses of a C5 inhibitor (e.g., an anti-C5 antibody) described herein can further be evaluated in, e.g., Phase I dose escalation studies. See, e.g., van Gurp et al. (2008) *Am J Transplantation* 8(8):1711-1718; Hanouska et al. (2007) *Clin Cancer Res* 13(2, part 1):523-531; and Hetherington et al. (2006) *Antimicrobial Agents and Chemotherapy* 50(10): 3499-3500.

The terms "therapeutically effective amount" or "therapeutically effective dose," or similar terms used herein are intended to mean an amount of an agent (e.g., a C5 inhibitor) that will elicit the desired biological or medical response (e.g., an improvement in one or more symptoms of a complement-associated disorder). In some embodiments, a composition described herein contains a therapeutically effective amount of an anti-C5 antibody. In some embodiments, a composition described herein contains a therapeutically effective amount of a siRNA, which specifically binds to and promotes inactivation of C5 mRNA in a mammalian cell. In some embodiments, a composition described herein contains a therapeutically effective amount of an antibody, which specifically binds to C5a. In some embodiments, the composition contains any of the antibodies described herein and one or more (e.g., three, four, five, six, seven, eight, nine, 10, or 11 or more) additional therapeutic agents such that the composition as a whole is therapeutically effective. For example, a composition can contain an anti-C5 antibody described herein and an immunosuppressive agent, wherein the antibody and agent are each at a concentration that when combined are therapeutically effective for treating or preventing a complement-associated disorder in a subject.

Toxicity and therapeutic efficacy of such compositions can be determined by known pharmaceutical procedures in cell cultures or experimental animals (e.g., animal models of any of the complement-associated disorders described herein). These procedures can be used, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. A complement inhibitor (e.g., a C5 inhibitor such as an anti-C5 antibody, an anti-C5a antibody, or a nucleic acid that binds to and promotes the inactivation of C5 mRNA in a mammalian cell) that exhibits a high therapeutic index is preferred. While compositions that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets

such compounds to the site of affected tissue and to minimize potential damage to normal cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such an inhibitor lies generally within a range of circulating concentrations of the inhibitor that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For a C5 inhibitor (e.g., an anti-C5 antibody or an anti-C5a antibody) used as described herein (e.g., for treating or preventing a complement-associated disorder), the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography or by ELISA.

In some embodiments, the methods can be performed in conjunction with other therapies for complement-associated disorders. For example, the composition can be administered to a subject at the same time, prior to, or after, plasmapheresis, IVIG therapy, plasma infusion, or plasma exchange. See, e.g., Appel et al. (2005) *J Am. Soc Nephrol.* 16:1392-1404. In some embodiments, a C5 inhibitor (e.g., an anti-C5 antibody or an anti-C5a antibody) described herein is not administered in conjunction with IVIG. In some embodiments, the composition can be administered to a subject at the same time, prior to, or after, a kidney transplant. Exemplary methods for transplanting an organ (e.g., a kidney) or tissue along with exemplary dosing schedules for an anti-C5 antibody are provided herein.

A "subject," as used herein, can be any mammal. For example, a subject can be a human (e.g., a patient), a non-human primate (e.g., monkey, baboon, or chimpanzee), a horse, a cow, a pig, a sheep, a goat, a dog, a cat, a rabbit, a guinea pig, a gerbil, a hamster, a rat, or a mouse. In some embodiments, the subject is an infant (e.g., a human infant). In some embodiments, the subject is a female.

As used herein, a subject "in need of prevention," "in need of treatment," or "in need thereof," refers to one, who by the judgment of an appropriate medical practitioner (e.g., a doctor, a nurse, or a nurse practitioner in the case of humans; a

veterinarian in the case of non-human mammals), would reasonably benefit from a given treatment (such as treatment with a composition comprising a complement inhibitor (e.g., a C5 inhibitor such as an anti-C5 antibody, an anti-C5a antibody, or a nucleic acid (e.g., an siRNA or antisense nucleic acid) that binds to and promotes the inactivation of a C5 mRNA in a mammalian cell).

As described above, the complement inhibitors (e.g., a C5 inhibitor such as an anti-C5 antibody) described herein can be used to treat a variety of complement-associated disorders such as, e.g., AP-associated disorders and/or CP-associated disorders. Such disorders include, without limitation, rheumatoid arthritis (RA); antiphospholipid antibody syndrome; lupus nephritis; ischemia-reperfusion injury; atypical hemolytic uremic syndrome (aHUS); typical or infectious hemolytic uremic syndrome (tHUS); dense deposit disease (DDD); neuromyelitis optica (NMO); multifocal motor neuropathy (MMN); multiple sclerosis (MS); macular degeneration (e.g., age-related macular degeneration (AMD)); hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome; thrombotic thrombocytopenic purpura (TTP); spontaneous fetal loss; Pauci-immune vasculitis; epidermolysis bullosa; recurrent fetal loss; and traumatic brain injury. (See, e.g., Holers (2008) *Immunological Reviews* 223:300-316 and Holers and Thurman (2004) *Molecular Immunology* 41:147-152.) In some embodiments, the complement-associated disorder is a complement-associated vascular disorder such as a cardiovascular disorder, myocarditis, a cerebrovascular disorder, a peripheral (e.g., musculoskeletal) vascular disorder, a renovascular disorder, a mesenteric/enteric vascular disorder, vasculitis, Henoch-Schönlein purpura nephritis, systemic lupus erythematosus-associated vasculitis, vasculitis associated with rheumatoid arthritis, immune complex vasculitis, Takayasu's disease, dilated cardiomyopathy, diabetic angiopathy, Kawasaki's disease (arteritis), venous gas embolus (VGE), and restenosis following stent placement, rotational atherectomy, and percutaneous transluminal coronary angioplasty (PTCA). (See, e.g., U.S. patent application publication no. 20070172483.) Additional complement-associated disorders include, without limitation, MG, CAD, dermatomyositis, Graves' disease, atherosclerosis, Alzheimer's disease, systemic inflammatory response sepsis, septic shock, spinal cord injury, glomerulonephritis, Hashimoto's thyroiditis, type I diabetes, psoriasis, pemphigus, AIHA, ITP,

Goodpasture syndrome, Degos disease, antiphospholipid syndrome (APS), and catastrophic APS (CAPS).

As used herein, a subject "at risk for developing a complement-associated disorder" (e.g., an AP-associated disorder or a CP-associated disorder) is a subject having one or more (e.g., two, three, four, five, six, seven, or eight or more) risk factors for developing the disorder. Risk factors will vary depending on the particular complement-associated disorder, but are well known in the art of medicine. For example, risk factors for developing DDD include, e.g., a predisposition to develop the condition, i.e., a family history of the condition or a genetic predisposition to develop the condition such as, e.g., one or more mutations in the gene encoding complement factor H (CFH), complement factor H-related 5 (CFHR5), and/or complement component C3 (C3). Such DDD-associated mutations as well as methods for determining whether a subject carries one or more of the mutations are known in the art and described in, e.g., Licht et al. (2006) *Kidney Int.* 70:42-50; Zipfel et al. (2006) "The role of complement in membranoproliferative glomerulonephritis," In: Complement and Kidney Disease, Springer, Berlin, pages 199-221; Ault et al. (1997) *J Biol. Chem.* 272:25168-75; Abrera-Abeleda et al. (2006) *J Med. Genet* 43:582-589; Poznansky et al. (1989) *J Immunol.* 143:1254-1258; Jansen et al. (1998) *Kidney Int.* 53:331-349; and Hegasy et al. (2002) *Am J Pathol* 161:2027-2034. Thus, a human at risk for developing DDD can be, e.g., one who has one or more DDD-associated mutations in the gene encoding CFH or one with a family history of developing the disease.

Risk factors for TTP are well known in the art of medicine and include, e.g., a predisposition to develop the condition, i.e., a family history of the condition or a genetic predisposition to develop the condition such as, e.g., one or more mutations in the ADAMTS13 gene. ADAMTS13 mutations associated with TTP are reviewed in detail in, e.g., Levy et al. (2001) *Nature* 413:488-494; Kokame et al. (2004) *Semin. Hematol.* 41:34-40; Licht et al. (2004) *Kidney Int.* 66:955-958; and Noris et al. (2005) *J. Am. Soc. Nephrol.* 16:1177-1183. Risk factors for TTP also include those conditions or agents that are known to precipitate TTP, or TTP recurrence, such as, but not limited to, cancer, bacterial infections (e.g., *Bartonella sp.* infections), viral infections (e.g., HIV and Kaposi's sarcoma virus), pregnancy, or surgery. See, e.g., Avery et al. (1998) *American Journal of Hematology* 58:148-149 and Tsai, *supra*).

TTP, or recurrence of TTP, has also been associated with the use of certain therapeutic agents (drugs) including, e.g., ticlopidine, FK506, corticosteroids, tamoxifen, or cyclosporin A (see, e.g., Gordon et al. (1997) *Seminars in Hematology* 34(2):140-147). Hereinafter, such manifestations of TTP may be, where appropriate, referred to as, e.g., "infection-associated TTP," "pregnancy-associated TTP," or "drug-associated TTP." Thus, a human at risk for developing TTP can be, e.g., one who has one or more TTP-associated mutations in the ADAMTS13 gene. A human at risk for developing a recurrent form of TTP can be one, e.g., who has had TTP and has an infection, is pregnant, or is undergoing surgery.

Risk factors for aHUS are well known in the art of medicine and include, e.g., a predisposition to develop the condition, i.e., a family history of the condition or a genetic predisposition to develop the condition such as, e.g., one or more mutations in complement Factor H (CFH), membrane cofactor protein (MCP; CD46), C4b-binding protein, complement factor B (CFB), or complement factor I (CFI). (See, e.g., Warwicker et al. (1998) *Kidney Int.* 53:836-844; Richards et al. (2001) *Am J Hum Genet* 68:485-490; Caprioli et al. (2001) *Am Soc Nephrol* 12:297-307; Neuman et al. (2003) *J Med Genet* 40:676-681; Richards et al. (2006) *Proc Natl Acad Sci USA* 100:12966-12971; Fremeaux-Bacchi et al. (2005) *J Am Soc Nephrol* 17:2017-2025; Esparza-Gordillo et al. (2005) *Hum Mol Genet* 14:703-712; Goicoechea de Jorge et al. (2007) *Proc Natl Acad Sci USA* 104(1):240-245; Blom et al. (2008) *J Immunol.* 180(9):6385-91; and Fremeaux-Bacchi et al. (2004) *J Medical Genet* 41:e84). (See also Kavanagh et al. (2006) *supra.*) Risk factors also include, e.g., infection with *Streptococcus pneumoniae*, pregnancy, cancer, exposure to anti-cancer agents (e.g., quinine, mitomycin C, cisplatin, or bleomycin), exposure to immunotherapeutic agents (e.g., cyclosporine, OKT3, or interferon), exposure to anti-platelet agents (e.g., ticlopidine or clopidogrel), HIV infection, transplantation, autoimmune disease, and combined methylmalonic aciduria and homocystinuria (cblC). See, e.g., Constantinescu et al. (2004) *Am J Kidney Dis* 43:976-982; George (2003) *Curr Opin Hematol* 10:339-344; Gottschall et al. (1994) *Am J Hematol* 47:283-289; Valavaara et al. (1985) *Cancer* 55:47-50; Miralbell et al. (1996) *J Clin Oncol* 14:579-585; Dragon-Durey et al. (2005) *J Am Soc Nephrol* 16:555-63; and Becker et al. (2004) *Clin Infect Dis* 39:S267-S275.

Risk factors for HELLP are well known in the art of medicine and include, e.g., multiparous pregnancy, maternal age over 25 years, Caucasian race, the occurrence of preeclampsia or HELLP in a previous pregnancy, and a history of poor pregnancy outcome. (See, e.g., Sahin et al. (2001) *Nagoya Med J* 44(3):145-152; Sullivan et al. (1994) *Am J Obstet Gynecol* 171:940-943; and Padden et al. (1999) *Am Fam Physician* 60(3):829-836.) For example, a pregnant, Caucasian woman who developed preeclampsia during a first pregnancy can be one at risk for developing HELLP syndrome during, or following, a second pregnancy.

Risk factors for CAD are well known in the art of medicine and include, e.g., conditions or agents that are known to precipitate CAD, or CAD recurrence, such as, but not limited to, neoplasms or infections (e.g., bacterial and viral infections). Conditions known to be associated with the development of CAD include, e.g., HIV infection (and AIDS), hepatitis C infection, *Mycoplasma pneumonia* infection, Epstein-Barr virus (EBV) infection, cytomegalovirus (CMV) infection, rubella, or infectious mononucleosis. Neoplasms associated with CAD include, without limitation, non-Hodgkin's lymphoma. Hereinafter, such manifestations of CAD may be, where appropriate, referred to as, e.g., "infection-associated CAD" or "neoplasm-associated CAD." Thus, a human at risk for developing CAD can be, e.g., one who has an HIV infection, rubella, or a lymphoma. See also, e.g., Gertz (2006) *Hematology* 1:19-23; Horwitz et al. (1977) *Blood* 50:195-202; Finland and Barnes (1958) *AMA Arch Intern Med* 191:462-466; Wang et al. (2004) *Acta Paediatr Taiwan* 45:293-295; Michaux et al. (1998) *Ann Hematol* 76:201-204; and Chang et al. (2004) *Cancer Genet Cytogenet* 152:66-69.

Risk factors for a thrombotic microangiopathy (TMA) are well known in the art of medicine and include, e.g., a medical history of aHUS, TTP, or other conditions that are associated with TMA such as lupus, cancers, disseminating intravascular coagulation and other coagulopathies, and pre-eclampsia. See, e.g., Copelovitch and Kaplan (2008) *Pediatr Nephrol* 23(10):1761-7.

Risk factors for PCH are well known in the art of medicine and include, e.g., conditions or agents that are known to precipitate PCH, or PCH recurrence, such as, but not limited to, neoplasms, infections (e.g., bacterial and viral infections), or certain immunizations (e.g., measles immunization). Conditions known to be associated with the development of PCH include, e.g., syphilis (a *Treponema*

palladium infection), measles, mumps, influenza virus infection, varicella-zoster virus infection, cytomegalovirus (CMV) infection, Epstein-Barr virus (EBV) infection, adenovirus infection, parvovirus B19 infection, Coxsackie A9 infection, *Haemophilus influenza* infection, *Mycoplasma pneumoniae* infection, and *Klebsiella pneumoniae* infection. See, e.g., Bunch et al. (1972) *Arch Dis Child* 47(252):299-300; Ziman et al. (2004) *Transfusion* 44(8):1127-1128; Sokol et al. (1984) *Acta Haematol* 72(4): 245-257; Papalia et al. (2000) *Br J Haematol* 109(2): 328-9; Sokol et al. (1982) *Acta Haematol* 68(4):268-277; and Bell et al. (1973) *Transfusion* 13(3):138-141. Neoplasms associated with PCH include, without limitation, both solid and hematopoietic neoplasms such as myelofibrosis, chronic lymphocytic leukemia (CLL), and non-Hodgkin's lymphoma. See, e.g., Sharara et al. (1994) *South Med J.* 87(3):397-9; Sivakumaran et al. (1999) *Br J Haematol* 105(1): 278-9; Breccia et al. (2004) *Eur J Haematol* 73(4):304-6; and Wynn et al. (1998) *Clin Lab Haematol* 20(6):373-5. Hereinafter, such manifestations of PCH may be, where appropriate, referred to as, e.g., "infection-associated PCH" or "neoplasm-associated PCH." Thus, a human at risk for developing PCH can be, e.g., one who has an EBV infection or a lymphoma.

Risk factors for MG are well known in the art of medicine and include, e.g., a predisposition to develop the condition, i.e., a family history of the condition or a genetic predisposition to develop the condition such as familial MG. For example, some HLA types are associated with an increased risk for developing MG. Risk factors for MG include the ingestion or exposure to certain MG-inducing drugs such as, but not limited to, D-penicillamine. See, e.g., Drosos et al. (1993) *Clin Exp Rheumatol.* 11(4):387-91 and Kaeser et al. (1984) *Acta Neurol Scand Suppl.* 100:39-47. As MG can be episodic, a subject who has previously experienced one or more symptoms of having MG can be at risk for relapse. Thus, a human at risk for developing MG can be, e.g., one who has a family history of MG and/or one who has ingested or been administered an MG-inducing drug such as D-penicillamine.

As used herein, a subject "at risk for developing CAPS" is a subject having one or more (e.g., two, three, four, five, six, seven, or eight or more) risk factors for developing the disorder. Approximately 60% of the incidences of CAPS are preceded by a precipitating event such as an infection. Thus, risk factors for CAPS include those conditions known to precipitate CAPS such as, but not limited to, certain

cancers (e.g., gastric cancer, ovarian cancer, lymphoma, leukemia, endometrial cancer, adenocarcinoma, and lung cancer), pregnancy, puerperium, transplantation, primary APS, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), surgery (e.g., eye surgery), and certain infections. Infections include, e.g., parvovirus B19 infection and hepatitis C infection. Hereinafter, such manifestations of CAPS may be referred to as, e.g., "cancer-associated CAPS," "transplantation-associated CAPS," "RA-associated CAPS," "infection-associated CAPS," or "SLE-associated CAPS." See, e.g., Soltész et al. (2000) *Haematologia (Budep)* 30(4):303-311; Ideguchi et al. (2007) *Lupus* 16(1):59-64; Manner et al. (2008) *Am J Med. Sci.* 335(5):394-7; Miesbach et al. (2006) *Autoimmune Rev.* 6(2):94-7; Gómez-Puerta et al. (2006) *Autoimmune Rev.* 6(2):85-8; Gómez-Puerta et al. (2006) *Semin. Arthritis Rheum.* 35(5):322-32; Kasamon et al. (2005) *Haematologia* 90(3):50-53; Atherson et al. (1998) *Medicine* 77(3):195-207; and Canpolat et al. (2008) *Clin Pediatr* 47(6):593-7. Thus, a human at risk for developing CAPS can be, e.g., one who has primary CAPS and/or a cancer that is known to be associated with CAPS.

From the above it will be clear that subjects "at risk for developing a complement-associated disorder" (e.g., an AP-associated disorder or a CP-associated disorder) are not all the subjects within a species of interest.

A subject "suspected of having a complement-associated disorder" (e.g., an alternative complement pathway-associated disorder) is one having one or more (e.g., two, three, four, five, six, seven, eight, nine, or 10 or more) symptoms of the disease. Symptoms of these disorders will vary depending on the particular disorder, but are known to those of skill in the art of medicine. For example, symptoms of DDD include, e.g.: one or both of hematuria and proteinuria; acute nephritic syndrome; drusen development and/or visual impairment; acquired partial lipodystrophy and complications thereof; and the presence of serum C3 nephritic factor (C3NeF), an autoantibody directed against C3bBb, the C3 convertase of the alternative complement pathway. (See, e.g., Appel et al. (2005), *supra*). Symptoms of aHUS include, e.g., severe hypertension, proteinuria, uremia, lethargy/fatigue, irritability, thrombocytopenia, microangiopathic hemolytic anemia, and renal function impairment (e.g., acute renal failure). Symptoms of TTP include, e.g., microthrombi, thrombocytopenia, fever, low ADAMTS13 metalloproteinase expression or activity, fluctuating central nervous system abnormalities, renal failure, microangiopathic

hemolytic anemia, bruising, purpura, nausea and vomiting (e.g., resulting from ischemia in the GI tract or from central nervous system involvement), chest pain due to cardiac ischemia, seizures, and muscle and joint pain. Symptoms of RA can include, e.g., stiffness, swelling, fatigue, anemia, weight loss, fever, and often, crippling pain. Some common symptoms of rheumatoid arthritis include joint stiffness upon awakening that lasts an hour or longer; swelling in a specific finger or wrist joints; swelling in the soft tissue around the joints; and swelling on both sides of the joint. Swelling can occur with or without pain, and can worsen progressively or remain the same for years before progressing. Symptoms of HELLP are known in the art of medicine and include, e.g., malaise, epigastric pain, nausea, vomiting, headache, right upper quadrant pain, hypertension, proteinuria, blurred vision, gastrointestinal bleeding, hypoglycemia, paresthesia, elevated liver enzymes/liver damage, anemia (hemolytic anemia), and low platelet count, any of which in combination with pregnancy or recent pregnancy. (See, e.g., Tomsen (1995) *Am J Obstet Gynecol* 172:1876-1890; Sibai (1986) *Am J Obstet Gynecol* 162:311-316; and Padden (1999), *supra*.)

Symptoms of CAPS are well known in the art of medicine and include, e.g., histopathological evidence of multiple small vessel occlusions; the presence of antiphospholipid antibodies (usually at high titer), vascular thromboses, severe multi-organ dysfunction, malignant hypertension, acute respiratory distress syndrome, disseminated intravascular coagulation, microangiopathic hemolytic anemia, schistocytes, and thrombocytopenia. CAPS can be distinguished from APS in that patients with CAPS generally present with severe multiple organ dysfunction or failure, which is characterized by rapid, diffuse small vessel ischemia and thromboses predominantly affecting the parenchymal organs. In contrast, APS is associated with single venous or arterial medium-to-large blood vessel occlusions. Symptoms of MG include, e.g., fatigability and a range of muscle weakness-related conditions including: ptosis (of one or both eyes), diplopia, unstable gait, depressed or distorted facial expressions, and difficulty chewing, talking, or swallowing. In some instances, a subject can present with partial or complete paralysis of the respiratory muscles. Symptoms of CAD include, e.g., pain, fever, pallor, anemia, reduced blood flow to the extremities (e.g., with gangrene), and renal disease or acute renal failure. In some embodiments, the symptoms can occur following exposure to cold temperatures.

From the above it will be clear that subjects "suspected of having a complement-associated disorder" are not all the subjects within a species of interest.

In some embodiments, the methods can include identifying the subject as one having, suspected of having, or at risk for developing, a complement-associated disorder in a subject. Suitable methods for identifying the subject are known in the art. For example, suitable methods (e.g., sequencing techniques or use of microarrays) for determining whether a human subject has a DDD-associated mutation in a CFH, CFHR5, or C3 gene are described in, e.g., Licht et al. (2006) *Kidney Int.* 70:42-50; Zipfel et al. (2006), *supra*; Ault et al. (1997) *J Biol. Chem.* 272:25168-75; Abrera-Abeleda et al. (2006) *J Med Genet* 43:582-589; Poznansky et al. (1989) *J Immunol.* 143:1254-1258; Jansen et al. (1998) *Kidney Int.* 53:331-349; and Hegasy et al. (2002) *Am J Pathol* 161:2027-2034. Methods for detecting the presence of characteristic DDD-associated electron-dense deposits are also well known in the art. For example, a medical practitioner can obtain a tissue biopsy from the kidney of a patient and subject the tissue to electron microscopy. The medical practitioner may also examine the tissue by immunofluorescence to detect the presence of C3 using an anti-C3 antibody and/or light microscopy to determine if there is membranoproliferative glomerulonephritis. See, e.g., Walker et al. (2007) *Mod. Pathol.* 20:605-616 and Habib et al. (1975) *Kidney Int.* 7:204-215. In some embodiments, the identification of a subject as one having DDD can include assaying a blood sample for the presence of C3NeF. Methods for detecting the presence of C3NeF in blood are described in, e.g., Schwertz et al. (2001) *Pediatr Allergy Immunol.* 12:166-172.

In some embodiments, the medical practitioner can determine whether there is increased complement activation in a subject's serum. Indicia of increased complement activation include, e.g., a reduction in CH50, a decrease in C3, and an increase in C3dg/C3d. See, e.g., Appel et al. (2005), *supra*. In some embodiments, a medical practitioner can examine a subject's eye for evidence of the development of drusen and/or other visual pathologies such as AMD. For example, a medical practitioner can use tests of retinal function such as, but not limited to, dark adaptation, electroretinography, and electrooculography (see, e.g., Colville et al. (2003) *Am J Kidney Dis.* 42:E2-5).

Methods for identifying a subject as one having, suspected of having, or at risk for developing, TTP are also known in the art. For example, Miyata et al. describe a variety of assays for measuring ADAMTS13 activity in a biological sample obtained from a subject (*Curr Opin Hematol* (2007) 14(3):277-283). Suitable ADAMTS13 activity assays, as well as phenotypically normal ranges of ADAMTS13 activity in a human subject, are described in, e.g., Tsai (2003) *J. Am. Soc. Nephrol* 14:1072-1081; Furlan et al. (1998) *New Engl J Med.* 339:1578-1584; Matsumoto et al. (2004) *Blood* 103:1305-1310; and Mori et al. (2002) *Transfusion* 42:572-580. Methods for detecting the presence of inhibitors of ADAMTS13 (e.g., autoantibodies that bind to ADAMTS13) in a biological sample obtained from a subject are known in the art. For example, a serum sample from a patient can be mixed with a serum sample from a subject without TTP to detect the presence of anti-ADAMTS13 antibodies. In another example, immunoglobulin protein can be isolated from patient serum and used in *in vitro* ADAMTS13 activity assays to determine if an anti-ADAMTS13 antibody is present. See, e.g., Dong et al. (2008) *Am J Hematol.* 83(10):815-817. In some embodiments, risk of developing TTP can be determined by assessing whether a patient carries one or more mutations in the ADAMTS13 gene. Suitable methods (e.g., nucleic acid arrays or DNA sequencing) for detecting a mutation in the ADAMTS13 gene are known in the art and described in, e.g., Levy et al., *supra*; Kokame et al., *supra*; Licht et al., *supra*; and Noris et al., *supra*.

In addition, methods for identifying a subject as one having, suspected of having, or at risk for developing aHUS are known in the art. For example, laboratory tests can be performed to determine whether a human subject has thrombocytopenia, microangiopathic hemolytic anemia, or acute renal insufficiency. Thrombocytopenia can be diagnosed by a medical professional as one or more of: (i) a platelet count that is less than 150,000/mm^3 (e.g., less than 60,000/mm^3); (ii) a reduction in platelet survival time, reflecting enhanced platelet disruption in the circulation; and (iii) giant platelets observed in a peripheral smear, which is consistent with secondary activation of thrombocytopoiesis. Microangiopathic hemolytic anemia can be diagnosed by a medical professional as one or more of: (i) hemoglobin concentrations that are less than 10 mg/dL (e.g., less than 6.5 mg/dL); (ii) increased serum lactate dehydrogenase (LDH) concentrations (>460 U/L); (iii) hyperbilirubinemia, reticulocytosis, circulating free hemoglobin, and low or undetectable haptoglobin concentrations; and

(iv) the detection of fragmented red blood cells (schistocytes) with the typical aspect of burr or helmet cells in the peripheral smear together with a negative Coombs test. (See, e.g., Kaplan et al. (1992) "Hemolytic Uremic Syndrome and Thrombotic Thrombocytopenic Purpura," Informa Health Care (ISBN 0824786637) and Zipfel (2005) "Complement and Kidney Disease," Springer (ISBN 3764371668).)

A subject can also be identified as having aHUS by evaluating blood concentrations of C3 and C4 as a measure of complement activation or dysregulation. In addition, as is clear from the foregoing disclosure, a subject can be identified as having genetic aHUS by identifying the subject as harboring one or more mutations in a gene associated with aHUS such as CFI, CFB, CFH, or MCP (*supra*). Suitable methods for detecting a mutation in a gene include, e.g., DNA sequencing and nucleic acid array techniques. (See, e.g., Breslin et al. (2006) *Clin Am Soc Nephrol* 1:88-99 and Goicoechea de Jorge et al. (2007) *Proc Natl Acad Sci USA* 104:240-245.)

Symptoms characteristic of TMA include, e.g., fever, microangiopathic hemolytic anemia (schistocytes in a blood smear), renal failure, thrombocytopenia, and neurological manifestations.

Methods for diagnosing a subject as one having, suspected of having, or at risk for developing, RA are also known in the art of medicine. For example, a medical practitioner can examine the small joints of the hands, wrists, feet, and knees to identify inflammation in a symmetrical distribution. The practitioner may also perform a number of tests to exclude other types of joint inflammation including arthritis due to infection or gout. In addition, rheumatoid arthritis is associated with abnormal antibodies in the blood circulation of afflicted patients. For example, an antibody referred to as "rheumatoid factor" is found in approximately 80% of patients. In another example, anti-citrulline antibody is present in many patients with rheumatoid arthritis and thus it is useful in the diagnosis of rheumatoid arthritis when evaluating patients with unexplained joint inflammation. See, e.g., van Venrooij et al. (2008) *Ann NY Acad Sci* 1143:268-285 and Habib et al. (2007) *Immunol Invest* 37(8):849-857. Another antibody called "the antinuclear antibody" (ANA) is also frequently found in patients with rheumatoid arthritis. See, e.g., Benucci et al. (2008) *Clin Rheumatol* 27(1):91-95; Julkunen et al. (2005) *Scan J Rheumatol* 34(2):122-124; and Miyawaki et al. (2005) *J Rheumatol* 32(8):1488-1494.

A medical practitioner can also examine red blood cell sedimentation rate to help in diagnosing RA in a subject. The sedimentation rate can be used as a crude measure of the inflammation of the joints and is usually faster during disease flares and slower during remissions. Another blood test that can be used to measure the degree of inflammation present in the body is the C-reactive protein.

Furthermore, joint x-rays can also be used to diagnose a subject as having rheumatoid arthritis. As RA progresses, the x-rays can show bony erosions typical of rheumatoid arthritis in the joints. Joint x-rays can also be helpful in monitoring the progression of disease and joint damage over time. Bone scanning, a radioactive test procedure, can demonstrate the inflamed joints.

Methods for identifying a subject as one having, suspected of having, or at risk for developing, HELLP are known in the art of medicine. Hallmark symptoms of HELLP syndrome include hemolysis, elevated liver enzymes, and low platelet count. Thus, a variety of tests can be performed on blood from a subject to determine the level of hemolysis, the concentration of any of a variety of liver enzymes, and the platelet level in the blood. For example, the presence of schistocytes and/or elevated free hemoglobin, bilirubin, or serum LDH levels is an indication of intravascular hemolysis. Routine laboratory testing can be used to determine the platelet count as well as the blood level of liver enzymes such as, but not limited to, aspartate aminotransferase (AST) and alanine transaminase (ALT). Suitable methods for identifying a subject as having HELLP syndrome are also described in, e.g., Sibai et al. (1993), *supra*; Martin et al. (1990), *supra*; Padden (1999), *supra*; and Gleicher and Buttino (1998) "Principles & Practice of Medical Therapy in Pregnancy," 3rd Edition, Appleton & Lange (ISBN 083857677X).

Suitable methods for identifying the subject as having MG can be qualitative or quantitative. For example, a medical practitioner can examine the status of a subject's motor functions using a physical examination. Other qualitative tests include, e.g., an ice-pack test, wherein an ice pack is applied to a subject's eye (in a case of ocular MG) to determine if one or more symptoms (e.g., ptosis) are improved by cold (see, e.g., Sethi et al. (1987) *Neurology* 37(8):1383-1385). Other tests include, e.g., the "sleep test," which is based on the tendency for MG symptoms to improve following rest. In some embodiments, quantitative or semi-quantitative tests can be employed by a medical practitioner to determine if a subject has, is suspected

of having, or is at risk for developing, MG. For example, a medical practitioner can perform a test to detect the presence or amount of MG-associated autoantibodies in a serum sample obtained from a subject. MG-associated autoantibodies include, e.g., antibodies that bind to, and modulate the activity of, acetylcholine receptor (AChR), muscle-specific receptor tyrosine kinase (MuSK), and/or striational protein. (See, e.g., Conti-Fine et al. (2006), *supra*). Suitable assays useful for detecting the presence or amount of an MG-associated antibody in a biological sample are known in the art and described in, e.g., Hoch et al. (2001) *Nat Med* 7:365-368; Vincent et al. (2004) *Semin Neurol.* 24:125-133; McConville et al. (2004) *Ann. Neurol.* 55:580-584; Boneva et al. (2006) *J Neuroimmunol.* 177:119-131; and Romi et al. (2005) *Arch Neurol.* 62:442-446.

Additional methods for diagnosing MG include, e.g., electrodiagnostic tests (e.g., single-fiber electromyography) and the Tensilon (or edrophonium) test, which involves injecting a subject with the acetylcholinesterase inhibitor edrophonium and monitoring the subject for an improvement in one or more symptoms. See, e.g., Pascuzzi (2003) *Semin Neurol* 23(1):83-88; Katirji et al. (2002) *Neurol Clin* 20:557-586; and "Guidelines in Electrodiagnostic Medicine. American Association of Electrodiagnostic Medicine," *Muscle Nerve* 15:229-253.

A subject can be identified as having CAD using an assay to detect the presence or amount (titer) of agglutinating autoantibodies that bind to the I antigen on red blood cells. The antibodies can be monoclonal (e.g., monoclonal IgM or IgA) or polyclonal. Suitable methods for detecting these antibodies are described in, e.g., Christenson and Dacie (1957) *Br J Haematol* 3:153-164 and Christenson et al. (1957) *Br J Haematol* 3:262-275. A subject can also be diagnosed as having CAD using one or more of a complete blood cell count (CBC), urinalysis, biochemical studies, and a Coombs test to test for hemolysis in blood. For example, biochemical studies can be used to detect elevated lactase dehydrogenase levels, elevated unconjugated bilirubin levels, low haptoglobin levels, and/or the presence of free plasma hemoglobin, all of which can be indicative of acute hemolysis. Other tests that can be used to detect CAD include detecting complement levels in the serum. For example, due to consumption during the acute phase of hemolysis, measured plasma complement levels (e.g., C2, C3, and C4) are decreased in CAD.

Typical (or infectious) HUS, unlike aHUS, is often identifiable by a prodrome of diarrhea, often bloody in nature, which results from infection with a shiga-toxin producing microorganism. A subject can be identified as having typical HUS when shiga toxins and/or serum antibodies against shiga toxin or LPS are detected in the stool of an individual. Suitable methods for testing for anti-shiga toxin antibodies or LPS are known in the art. For example, methods for detecting antibodies that bind to shiga toxins Stx1 and Stx2 or LPS in humans are described in, e.g., Ludwig et al. (2001) *J Clin Microbiol* 39(6):2272-2279.

Symptoms of this condition are known to those of skill in the art of medicine and include, e.g., pain, fever, pallor, icterus, urticarial dermal eruption, hemoglobinuria, hemoglobinemia, anemia, and renal disease or acute renal failure. In some embodiments, the symptoms can occur following exposure to cold temperatures.

In some embodiments, the methods can include identifying the subject as one having, suspected of having, or at risk for developing, PCH. Suitable methods for identifying the subject are known in the art. For example, a subject can be diagnosed as having PCH using a Donath-Landsteiner test, which is an assay to detect the presence of the Donath-Landsteiner antibody in a subject's serum. The procedure involves incubating three samples – (1) the subject's serum; (2) normal serum; and (3) a mix of the subject's serum and normal serum – with P-antigen expressing red blood cells at 0 to 4°C. Next, the sample is warmed to 37°C and visually inspected for hemolysis. If the Donath-Landsteiner antibody is present, hemolysis should occur in samples (1) and (3), but not in (2). See, e.g., Funato et al. (2007) *Eur J Haematol* 79(5):462; Win et al. (2005) Transfus Med. 15(3):254; Sokol et al. (1998) *Immunohematology* 14(3):109-12; Eder (2005) *Immunohematology* 21(2):56-62; and Dacie et al. (1957) *Br J Haematol* 3:77-87. A subject can also be diagnosed as having PCH using one or more of a complete blood cell count (CBC), urinalysis, biochemical studies, and a Coombs test. For example, biochemical studies can be used to detect elevated lactase dehydrogenase levels, elevated unconjugated bilirubin levels, low haptoglobin levels, and/or the presence of free plasma hemoglobin, all of which can be indicative of acute hemolysis. Other tests that can be used to detect PCH include detecting complement levels in the serum. For example, due to consumption during the acute phase of hemolysis, measured plasma complement levels (e.g., C2, C3, and C4) are decreased in PCH. See also, e.g., Nordhagen et al. (1984) *Acta Paediatr*

Scand 73(2):258-262; Lindgren et al. (1985) *Transfusion* 25(2):142-4; Nordhagen et al. (1991) *Transfusion* 31(2):190-1; and Garratty (2001) *Transfusion* 41(8):1073-4.

In some embodiments, the composition can be administered to a subject prophylactically to prevent, or prevent relapse or recurrence of, PCH. For example, a subject who previously had an advanced *Mycoplasma* infection or who is newly diagnosed with a PCH-associated neoplasm can be administered a composition described herein to prevent, lessen the severity of, or prevent a recurrence of PCH.

In some embodiments, a C5 inhibitor (e.g., an anti-C5 antibody) described herein can be administered to a subject as a monotherapy. Alternatively, as described above, the antibody can be administered to a subject as a combination therapy with another treatment, e.g., another treatment for DDD, TTP, aHUS, RA, HELLP, MG, CAD, CAPS, tHUS, Degos disease, or any other complement-associated disorder described herein. For example, the combination therapy can include administering to the subject (e.g., a human patient) one or more additional agents (e.g., anti-coagulants, anti-hypertensives, or corticosteroids) that provide a therapeutic benefit to the subject who has, or is at risk of developing, DDD. In some embodiments, the combination therapy can include administering to the subject (e.g., a human patient) a C5 inhibitor (e.g., an anti-C5 antibody or an anti-C5a antibody) and an immunosuppressive agent such as Remicade® for use in treating RA. In some embodiments, the C5 inhibitor and the one or more additional active agents are administered at the same time. In other embodiments, a C5 inhibitor is administered first in time and the one or more additional active agents are administered second in time. In some embodiments, the one or more additional active agents are administered first in time and the C5 inhibitor is administered second in time.

A C5 inhibitor (e.g., an anti-C5 antibody) described herein can replace or augment a previously or currently administered therapy. For example, upon treating with an anti-C5 antibody, administration of the one or more additional active agents can cease or diminish, e.g., be administered at lower levels. In some embodiments, administration of the previous therapy can be maintained. In some embodiments, a previous therapy will be maintained until the level of the C5 inhibitor (e.g., anti-C5 antibody or an anti-C5a antibody) reaches a level sufficient to provide a therapeutic effect. The two therapies can be administered in combination.

In some embodiments, a C5 inhibitor can be administered to a patient chronically. For example, a patient chronically treated with a complement-inhibiting agent (e.g., a C5 inhibitor or a C5a inhibitor) can be treated for a period of greater than or equal to 2 weeks (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 weeks; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, or 12 years or for the remainder of the patient's life) with the agent in an amount and with a dosing frequency that are sufficient to maintain a concentration of the agent in the patient's blood that inhibits or substantially inhibits systemic complement activity in the patient. To maintain systemic complement inhibition in a patient, a C5 inhibitor can be chronically administered to the patient, e.g., once a week, once every two weeks, twice a week, once a day, once a month, or once every three weeks. In some embodiments of any of the methods described herein, the C5 inhibitor can be administered to a patient in an amount and with a frequency of administration effective to maintain a concentration of at least: 0.7 (e.g., at least 0.8, 0.9, one, two, three, four, five, six, seven, eight, nine, or 10 or more) bivalent C5 inhibitor (e.g., a whole antibody) molecule(s) per every C5 molecule in the patient's blood; or 1.5 (e.g., at least 1.6, 1.7, 1.8, 1.9, two, three, four, five, six, seven, eight, nine, or 10 or more) monovalent C5 inhibitor (e.g., a single chain anti-C5 antibody or a Fab fragment of the antibody) molecule(s) per every C5 molecule in the patient's blood. For example, in some embodiments a monovalent anti-C5 antibody (e.g., a single chain antibody or a Fab antibody fragment) can be administered to a patient in an amount and with a frequency effective to maintain a concentration of at least 1.5 (e.g., at least 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, or 7 or more) monovalent anti-C5 antibodies per C5 molecule in the blood. In some embodiments of any of the methods described herein, an anti-C5 antibody is administered to the patient in an amount and with a frequency that are effective to maintain a concentration of at least 40 (e.g., 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 80, 85, 90, 95, 100, 110, or 120 or more) μg of the antibody per milliliter of the patient's blood. Exemplary chronic dosing strategies are described herein (see, e.g., Tables 1 and 2).

In some embodiments, the C5 inhibitor (or C5a inhibitor) can be administered to a subject even after one or more symptoms have been ameliorated. Monitoring a subject (e.g., a human patient) for an improvement in a complement-associated disorder, as defined herein, means evaluating the subject for a change in a disease parameter, e.g., an improvement in one or more symptoms of the disease. Such symptoms include any of the symptoms of complement-associated disorders described herein. In some embodiments, the evaluation is performed at least 1 hour, e.g., at least 2, 4, 6, 8, 12, 24, or 48 hours, or at least 1 day, 2 days, 4 days, 10 days, 13 days, 20 days or more, or at least 1 week, 2 weeks, 4 weeks, 10 weeks, 13 weeks, 20 weeks or more, after an administration. The subject can be evaluated in one or more of the following periods: prior to beginning of treatment; during the treatment; or after one or more elements of the treatment have been administered. Evaluating can include evaluating the need for further treatment, e.g., evaluating whether a dosage, frequency of administration, or duration of treatment should be altered. It can also include evaluating the need to add or drop a selected therapeutic modality, e.g., adding or dropping any of the treatments for any of the complement-associated disorders described herein.

In some embodiments, the complement inhibitor can be chronically administered to a patient in need thereof in an amount and with a frequency that are effective to reduce and maintain serum hemolytic activity at less than or equal to 20 (e.g., 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, or even below 5) %. *See*, e.g., Hill et al. (2005) *Blood* 106(7):2559. In some embodiments, the complement inhibitor can be administered to a patient in an amount and with a frequency that are effective to maintain serum lactate dehydrogenase (LDH) levels at within at least 20 (e.g., 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, or even below 5) % of the normal range for LDH. *See* Hill et al. (2005) *supra*. In some embodiments, the complement inhibitor is administered to the patient in an amount and with a frequency that are effective to maintain a serum LDH level less than 550 (e.g., less than 540, 530, 520, 510, 500, 490, 480, 470, 450, 440, 430, 420, 410, 400, or less than 300) IU/L. In some embodiments, administration (e.g., chronic administration) of a C5 inhibitor (e.g., an anti-C5 antibody such as eculizumab) or a C5a inhibitor (e.g., an anti-C5a antibody) results in amelioration of one or more of a patient's symptoms to within 40 (e.g., 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21,

20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or even 1) % of its normal level or value. For example, in some embodiments, the elevated blood pressure in an aHUS patient treated (e.g., chronically treated) with an anti-C5 antibody can be reduced to a level that is within 40% of the level that is normal for a person of the patient's age, race, height, weight, sex, and physical health.

In some embodiments, the complement inhibitor (e.g., a C5 inhibitor or C5a inhibitor) is administered to a subject even after the patient has entered clinical remission. Determining clinical remission of a complement-associated disorder is well within the skill set of the skilled artisan in medicine. For example, elements determinative of clinical remission for aHUS are described in, e.g., Nürnberger et al. (2009) *N Engl J Med* 360(5):542-544. Clinical remission for CAPS is described in, e.g., Manner et al. (2008) *Am J Med Sci* 335(5):394-397.

The disclosure also provides methods for allogeneic organ or tissue transplantation. The method includes transplanting an organ or tissue into a patient in need thereof, wherein prior to and following the transplanting a C5 inhibitor is administered to the patient in an amount and with a frequency effective to substantially inhibit systemic complement activity in the patient. As described herein, the C5 inhibitor (e.g., the anti-C5 antibody) can be administered in an amount and with a frequency to maintain a concentration of at least one C5 inhibitor molecule (e.g., at least one anti-C5 antibody) per C5 molecule in the patient's blood. In some embodiments, a monovalent anti-C5 antibody (e.g., a single chain antibody or a Fab antibody fragment) can be administered to a patient in an amount with a frequency effective to maintain a concentration of at least 1.5 (e.g., at least 1.6, 1.7, 1.8, 1.9, 2, 3, 4, 5, 6, or 7 or more) monovalent anti-C5 antibodies per C5 molecule in the blood. In some embodiments, the C5 inhibitor (e.g., the anti-C5 antibody) can be administered to the patient in an amount and with a frequency to maintain a concentration of at least at least 40 (e.g., 41, 42, 43, 44, 45, 46, 47, 48, 48, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 80, 85, 90, 95, 100, 110, or 120 or more) μg of the inhibitor (e.g., the anti-C5 antibody) in the patient's blood. In some embodiments, at least 800 (e.g., at least 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody (e.g., eculizumab) is administered to the patient less than 24 (e.g., less than 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12,

11, 10, 9, 8, 7, 6, 5, 4, 3, or less than 2) hours prior to transplanting the organ or tissue to the patient. In some embodiments, the methods can also include, prior to the transplanting, contacting (e.g., soaking) the donor organ or tissue with a C5 inhibitor (e.g., an anti-C5 antibody such as eculizumab) for an amount of time and under conditions that inhibit complement activation in the organ or tissue upon transplantation. The organ can be, e.g., skin, a kidney, heart, lung, limb (e.g., finger or toe), or liver. In some embodiments, the methods can include administering a C5 inhibitor (e.g., an anti-C5 antibody) to the donor patient prior to removal of the organ or tissue for transplant. The patient can have, be at risk for developing, or be suspected of having aHUS. In some embodiments, at least 700 (e.g., at least 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody is administered to the patient less than 24 (e.g., less than 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or less than 2) hours following the transplanting. In some embodiments, the anti-C5 antibody is chronically administered to the patient following the transplanting. For example, the anti-C5 antibody can be chronically administered to the patient for at least 9 weeks (e.g., 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 weeks; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, or 12 years or for the remainder of the patient's life) under the following dosing schedule: at least 700 (e.g., at least 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody less than 24 hours after transplanting the organ or tissue; at least 700 (e.g., at least 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody once per week for four weeks after the initial post-transplant dose; at least 700 (e.g., at least 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody once during the fifth week; and at least 700 (e.g., at least 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910,

920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, or 1200 or more) mg of the anti-C5 antibody bi-weekly thereafter for the remainder of the dosing schedule. In a preferred embodiment, the anti-C5 antibody is administered such that the first four doses are at least 900 mg of the antibody; 1200 mg is administered on the fifth week; and 1200 mg is administered to the patient bi-weekly thereafter for the remainder of the chronic treatment schedule. Addition exemplary dosing schedules are provided in Tables 1 and 2.

In some embodiments, the methods include administering an immunosuppressant to the patient. Suitable immunosuppressants for use in the methods include, but are not limited to, ATG or ALG, OKT3, daclizumab, basiliximab, corticosteroids, 15-deoxyspergualin, cyclosporins, tacrolimus, azathioprine, methotrexate, mycophenolate mofetil, 6-mercaptopurine, bredinin, brequinar, leflunamide, cyclophosphamide, sirolimus, anti-CD4 monoclonal antibodies, CTLA4-Ig, anti-CD154 monoclonal antibodies, anti-LFAl monoclonal antibodies, anti-LFA-3 monoclonal antibodies, anti-CD2 monoclonal antibodies, and anti-CD45 antibodies.

Types of organs or tissues that can be transplanted using the methods described herein include, e.g., heart, kidney, lung, pancreas, liver, vascular tissue, eye, cornea, lens, skin, bone marrow, muscle, connective tissue, gastrointestinal tissue, nervous tissue, bone, stem cells, islets, cartilage, hepatocytes, and hematopoietic cells.

In some embodiments, the transplant methods will result in prolongation of the graft in the recipient patient by at least one month (e.g., three, four, five, six, seven, eight, nine, 10, 11, or 12 months or 1, 2, 3, 4, 5, 6, 7, 8, 9, or even 10 years or more).

***Ex vivo* approaches.** An *ex vivo* strategy for treating or preventing a complement-associated disorder (e.g., an AP-associated disorder or a CP-associated disorder) can involve transfecting or transducing one or more cells obtained from a subject with a polynucleotide encoding a complement inhibitor (e.g., an anti-C5 antibody, anti-C5a antibody, or a nucleic acid (e.g., a siRNA) that binds to and promotes inactivation of a C5 mRNA in a mammalian cell) described herein. For example, the cells can be transfected with a single vector encoding a heavy and light chain of an antibody that binds to C5 protein, or the cells can be transfected with a

first vector encoding a heavy chain and a second vector encoding a light chain of the antibody.

The transfected or transduced cells are then returned to the subject. The cells can be any of a wide range of types including, without limitation, hemopoietic cells (e.g., bone marrow cells, macrophages, monocytes, dendritic cells, T cells, or B cells), fibroblasts, epithelial cells, endothelial cells, keratinocytes, or muscle cells. Such cells can act as a source (e.g., sustained or periodic source) of the C5 inhibitor (e.g., anti-C5 antibody, anti-C5a antibody, or nucleic acid (above)) for as long as they survive in the subject. In some embodiments, the vectors and/or cells can be configured for inducible or repressible expression of the C5 inhibitor (see, e.g., Schockett et al. (1996) *Proc Natl Acad Sci USA* 93: 5173-5176 and U.S. Patent No. 7,056,897).

Preferably, the cells are obtained from the subject (autologous), but can potentially be obtained from a subject of the same species other than the subject (allogeneic).

Suitable methods for obtaining cells from a subject and transducing or transfecting the cells are known in the art of molecular biology. For example, the transduction step can be accomplished by any standard means used for *ex vivo* gene therapy, including calcium phosphate, lipofection, electroporation, viral infection (see above), and biolistic gene transfer. (See, e.g., Sambrook et al. (*supra*) and Ausubel et al. (1992) "Current Protocols in Molecular Biology," Greene Publishing Associates.) Alternatively, liposomes or polymeric microparticles can be used. Cells that have been successfully transduced can be selected, for example, for expression of the coding sequence or of a drug resistance gene.

Kits

The disclosure also features articles of manufacture or kits, which include a container with a label; and a composition containing one or more complement inhibitors described herein. For example, the kit can contain one or more of an anti-C5a antibody, an anti-C5 antibody, and a nucleic acid (e.g., an siRNA or antisense nucleic acid) that binds to and promotes inactivation of a C5 mRNA in a mammalian cell. The label indicates that the composition is to be administered to a subject (e.g., a human) having, suspected of having, or at risk for developing, a complement-

associated disorder (e.g., an AP- or CP-associated disorder) such as DDD, aHUS, TTP, HELLP, RA, AMD, tHUS, MG, CAD, PCH, CAPS, Degos disease, or any other complement pathway-associated disorder described herein. The kit can, optionally, include a means for administering the composition to the subject. For example, the kits can include one or more syringes.

In some embodiments, the kits can further include one or more additional active agents such as any of those described herein. For example, the kits can include one or more corticosteroids, anti-hypertensives, immunosuppressives, and anti-seizure agents.

The following examples are intended to illustrate, not limit, the invention.

Example 1

A human adult patient is identified by a medical practitioner as having an inherited form of aHUS. Once a week for four weeks the patient is administered a composition containing eculizumab at a dose of 900 mg. The patient then receives at least 1200 mg of eculizumab once during the fifth week and at least 1200 mg of eculizumab bi-weekly thereafter. The patient and medical practitioner observe a substantial improvement in at least two known symptoms of aHUS during the initial treatment. Eculizumab is chronically administered to the patient even after the medical practitioner determines that the aHUS is in remission.

Example 2

A human patient weighing around 25 kg is identified by a medical practitioner as having aHUS. Once a week for two weeks the patient is administered a composition containing eculizumab at a dose of at least 600 mg. The patient then receives at least 600 mg of eculizumab once during the third week and at least 600 mg of eculizumab bi-weekly thereafter. The patient and medical practitioner observe a substantial improvement in at least two known symptoms of aHUS during the initial treatment. Eculizumab is chronically administered to the patient even after the medical practitioner determines that the aHUS is in remission in order to prevent a recurrence of aHUS in the patient.

Example 3

A human patient weighing around 35 kg is identified by a medical practitioner as having CAPS. Once a week for two weeks the patient is administered a composition containing eculizumab at a dose of at least 600 mg. The patient then receives at least 900 mg of eculizumab once during the third week and at least 900 mg of eculizumab bi-weekly thereafter. The patient and medical practitioner observe a substantial improvement in at least two known symptoms of CAPS during the initial treatment. Eculizumab is chronically administered to the patient even after the medical practitioner determines that the CAPS is in remission in order to prevent, or substantially reduce the likelihood of, a recurrence of CAPS in the patient.

Example 4

A human patient weighing around 7 kg is identified by a medical practitioner as having aHUS. The patient has TMA in her kidneys as a result of the aHUS. For one week the patient is administered a composition containing eculizumab at a dose of at least 300 mg. The patient then receives at least 300 mg of eculizumab once during the second week and at least 300 mg of eculizumab every three weeks thereafter. The patient and medical practitioner observe a substantial improvement in at least two known symptoms of aHUS during the initial treatment. The medical practitioner also observes that the TMA in the patient's kidneys resolves and no new TMA occurs while the patient is being chronically administered eculizumab. Eculizumab is chronically administered to the patient even after the medical practitioner determines that the aHUS is in remission in order to prevent, or substantially reduce the likelihood of, a recurrence of aHUS in the patient and any further damage to her kidneys that could result from recurrence.

Example 5

A human patient in need of a kidney transplant is intravenously administered eculizumab at a dose of 1200 mg less than 24 hours before the transplant operation. An allogeneic kidney is transplanted into the patient. Less than 24 hours after the kidney transplant, the patient is administered another 1200 mg of eculizumab. Once a week for four weeks following the first post-operation dose of eculizumab, the patient receives 900 mg of eculizumab. The patient receives 1200 mg of eculizumab on the

fifth week after the initial post-operation dose of eculizumab and then is maintained on a dosing schedule that includes 1200 mg of eculizumab bi-weekly thereafter. The medical practitioner observes a substantial improvement in the survival of the transplanted kidney in the patient.

While the present disclosure has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the disclosure. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present disclosure. All such modifications are intended to be within the scope of the disclosure.

What is claimed is:

1. A method for treating a complement-associated disorder, the method comprising chronically administering to a patient in need thereof a complement inhibitor in an amount and with a frequency that are effective to substantially inhibit systemic complement activity in the patient, wherein the patient has, is suspected of having, or is at risk for developing a complement-associated disorder and with the proviso that the complement-associated disorder is not paroxysmal nocturnal hemoglobinuria.

2. A method for transplanting an allogeneic organ or tissue, the method comprising transplanting an organ or tissue into a patient in need thereof, wherein prior to and following the transplanting a complement inhibitor is administered to the patient in an amount and with a frequency effective to substantially inhibit systemic complement activity in the patient.

3. The method of claim 2, wherein the organ or tissue is selected from the group consisting of a liver, a kidney, a heart, a lung, skin, eye, bone marrow, and vascular tissue.

4. The method of claim 2 or 3, wherein the patient has, is suspected of having, or is at risk for developing a complement-associated disorder.

5. The method of any one of claims 1 to 4, wherein the complement inhibitor is chronically administered to a patient in an amount and with a frequency that are effective to reduce serum hemolytic activity to less than or equal to 20% and to maintain the serum hemolytic activity at less than or equal to 20%.

6. The method of any one of claims 1 to 5, wherein the complement inhibitor is chronically administered to a patient in an amount and with a frequency that are effective to maintain a serum LDH level that is less than or equal to 500 IU/L.

7. The method of any one of claims 1 to 6, wherein the complement inhibitor is selected from the group consisting of a polypeptide, a polypeptide analog, a nucleic acid, a nucleic acid analog, and a small molecule.

8. The method of any one of claims 1 to 7, wherein the complement inhibitor is selected from the group consisting of soluble CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

9. The method of any one of claims 1 to 7, wherein the complement inhibitor inhibits the expression of a human complement component protein.

10. The method of any one of claims 1 to 7, wherein the complement inhibitor inhibits the cleavage of human complement component C5, C4, C3, or C2.

11. The method of claim 10, wherein the complement inhibitor inhibits the cleavage of complement component C5 into fragments C5a and C5b.

12. The method of claim 10 or 11, wherein the complement inhibitor is an antibody or antigen-binding fragment thereof that binds to a human complement component protein.

13. The method of claim 12, wherein the antibody or antigen-binding fragment thereof binds to a human complement component C5 protein.

14. The method of claim 13, wherein the antibody binds to the alpha chain of the complement component C5 protein.

15. The method of claim 13 or 14, wherein the antibody binds to the alpha chain of human complement component C5, and wherein the antibody (i) inhibits complement activation in a human body fluid, (ii) inhibits the binding of purified human complement component C5 to either human complement component C3b or

human complement component C4b, and (iii) does not bind to the human complement activation product free C5a.

16. The method of claim 13, wherein the antibody binds to the human complement component C5 protein having the amino acid sequence depicted in any one of SEQ ID NOs:1-26.

17. The method of claim 13, wherein the antibody binds to an isolated oligopeptide comprising an amino acid sequence corresponding to amino acid position 8 through amino acid position 12 of SEQ ID NO:5.

18. The method of claim 12 or 13, wherein the polypeptide comprises an antibody that binds to complement component C5 fragment C5b.

19. The method of any one of claims 12 to 18, wherein the antibody is a monoclonal antibody.

20. The method of any one of claims 12 to 19, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of a humanized antibody, a recombinant antibody, a diabody, a chimerized or chimeric antibody, a deimmunized human antibody, a fully human antibody, a single chain antibody, an Fv fragment, an Fd fragment, an Fab fragment, an Fab' fragment, and an $F(ab')_2$ fragment.

21. The method of claim 12 or 13, wherein the antibody is eculizumab.

22. The method of any one of claims 12, 13, or 21, wherein the antibody is administered to the patient in an amount and with a frequency to maintain a concentration of at least 0.7 anti-C5 antibody molecules per every C5 molecule in the patient's blood.

23. The method of any one of claims 12, 13, or 21, wherein the antibody is administered to the patient in an amount and with a frequency to maintain at least 50 μg of the antibody per milliliter of the patient's blood.

24. The method of claim 23, wherein the antibody is administered to the patient in an amount and with a frequency effective to maintain a concentration of at least 100 μg of the antibody per milliliter of the patient's blood.

25. The method of any one of claims 1 to 24, wherein the body weight of the patient is greater than or equal to 40 kg.

26. The method of any one of claims 12, 13, or 21 to 25, wherein the antibody is administered to the patient for at least 7 weeks under the following schedule:

at least 800 mg of the antibody, once per week for four consecutive weeks;

at least 800 mg of the antibody once during the fifth week; and

at least 800 mg of the antibody bi-weekly thereafter.

27. The method of claim 26, wherein the antibody is administered to the patient for at least 7 weeks under the following schedule:

at least 900 mg of the antibody, once per week for four consecutive weeks;

at least 1200 mg of the antibody once during the fifth week; and

at least 1200 mg of the antibody bi-weekly thereafter.

28. The method of any one of claims 1 to 24, wherein the body weight of the patient is less than 40 kg but greater than or equal to 30 kg.

29. The method of claim 28, wherein the antibody is chronically administered to the patient for at least 5 weeks under the following schedule:

at least 500 mg of the antibody, once per week for two consecutive weeks;

at least 700 mg of the antibody once during the third week; and

at least 700 mg of the antibody bi-weekly thereafter.

30. The method of claim 29, wherein the antibody is chronically administered to the patient for at least 5 weeks under the following schedule:

at least 600 mg of the antibody, once per week for two consecutive weeks;

at least 900 mg of the antibody once during the third week; and

at least 900 mg of the antibody bi-weekly thereafter.

31. The method of any one of claims 1 to 24, wherein the body weight of the patient is less than 30 kg, but is greater than or equal to 20 kg.

32. The method of claim 31, wherein the antibody is chronically administered to the patient for at least 5 weeks under the following schedule:

at least 500 mg of the antibody, once per week for two consecutive weeks;

at least 500 mg of the antibody once during the third week; and

at least 500 mg of the antibody bi-weekly thereafter.

33. The method of claim 32, wherein the antibody is chronically administered to the patient for at least 5 weeks under the following schedule:

at least 600 mg of the antibody, once per week for two consecutive weeks;

at least 600 mg of the antibody once during the third week; and

at least 600 mg of the antibody bi-weekly thereafter.

34. The method of any one of claims 1 to 24, wherein the body weight of the patient is less than 20 kg, but is greater than or equal to 10 kg.

35. The method of claim 34, wherein the antibody is chronically administered to the patient for at least 4 weeks under the following schedule:

at least 500 mg of the antibody once a week for one week;

at least 200 mg of the antibody once during the second week; and

at least 200 mg of the antibody bi-weekly thereafter.

36. The method of claim 36, wherein the antibody is chronically administered to the patient for at least 4 weeks under the following schedule:

at least 600 mg of the antibody once a week for one week;

at least 300 mg of the antibody once during the second week; and

at least 300 mg of the antibody bi-weekly thereafter.

37. The method of any one of claims 1 to 24, wherein the body weight of the patient is less than 10 kg, but is greater than or equal to 5 kg.

38. The method of claim 37, wherein the antibody is chronically administered to the patient for at least 5 weeks under the following schedule:

at least 200 mg of the antibody, once per week for one week;

at least 200 mg of the antibody once during the second week; and

at least 200 mg of the antibody once every three weeks thereafter.

39. The method of claim 38, wherein the antibody is chronically administered to the patient for at least 5 weeks under the following schedule:

at least 300 mg of the antibody, once per week for one week;

at least 300 mg of the antibody once during the second week; and

at least 300 mg of the antibody every three weeks thereafter.

40. The method of any one of claims 2 to 24, wherein the complement inhibitor is administered to the patient less than 24 hours prior to the transplanting.

41. The method of any one of claims 2 to 24 or 40, wherein the complement inhibitor is administered to the patient less than 12 hours prior to transplanting.

42. The method of any one of claims 2 to 24, 40, or 41, wherein the complement inhibitor is administered to the patient less than 24 hours following the transplanting of the organ or tissue.

43. The method of any one of claims 2 to 24, 40, 41, or 42, wherein the complement inhibitor is administered to the patient less than 12 hours following the transplanting of the organ or tissue.

44. The method of claim 2 to 4 or 40 to 43, wherein the complement inhibitor is a whole antibody that binds to complement component C5 and, prior to the transplanting, at least 900 mg of the antibody is administered to the patient.

45. The method of claim 44, wherein, prior to the transplanting, at least 1,200 mg of the antibody is administered to the patient.

46. The method of any one of claims 2 to 4 or 40 to 45, wherein the complement inhibitor is a whole antibody that binds to complement component C5 and, following the transplanting, at least 800 mg of the antibody is administered to the patient.

47. The method of claim 46, wherein following the transplanting, at least 900 mg of the antibody is administered to the patient.

48. The method of claim 46 or 47, wherein following the transplanting, the anti-C5 antibody is administered to the patient for at least 7 weeks under the following schedule:

at least 800 mg of the anti-C5 antibody less than 24 hours after transplanting the organ or tissue;

at least 800 mg of the anti-C5 antibody once per week for four weeks;

at least 800 mg of the anti-C5 antibody once during the fifth week; and

at least 800 mg of the anti-C5 antibody bi-weekly thereafter.

49. The method of any one of claims 46, 47, or 48, wherein following the transplanting, the anti-C5 antibody is administered to the patient for at least 7 weeks under the following schedule:

at least 1200 mg of the anti-C5 antibody less than 24 hours after transplanting the organ or tissue;

at least 900 mg of the anti-C5 antibody once per week for four weeks;

at least 1200 mg of the anti-C5 antibody once during the fifth week; and

at least 1200 mg of the anti-C5 antibody bi-weekly thereafter.

50. The method of any one of claims 26, 27, 29, 30, 32, 33, 35, 36, 38, 39, 48, or 49, wherein the antibody is administered to the patient for at least 21 weeks under the schedule.

51. The method of any one of claims 26, 27, 29, 30, 32, 33, 35, 36, 38, 39, 48, 49, or 50, wherein the antibody is administered to the patient for at least one year under the schedule.

52. The method of any one of claims 26, 27, 29, 30, 32, 33, 35, 36, 38, 39, or 48 to 51, wherein the inhibitor is administered to the patient for the remainder of the patient's life.

53. The method of any one of claims 1 to 25, 28, 31, 34, 37, or 40 to 47, wherein the inhibitor is chronically administered to the patient at least once per week.

54. The method of any one of claims 1 to 25, 28, 31, 34, 37, or 40 to 47, wherein the inhibitor is chronically administered to the patient at least once every two weeks.

55. The method of any one of claims 1 to 54, wherein the inhibitor is chronically administered to the patient for the remainder of the patient's life.

56. A method for treating thrombotic microangiopathy (TMA) in a patient who has, is suspected of having, or at risk of developing TMA, the method comprising administering to a patient in need thereof an inhibitor of human complement to thereby treat TMA in the patient.

57. A method for reducing the occurrence or severity of thrombotic microangiopathy (TMA) in a patient who has, is suspected of having, or at risk of developing TMA, the method comprising administering to a patient in need thereof an inhibitor of human complement to thereby reduce the occurrence or severity of TMA in the patient.

58. The method of claim 56 or 57, wherein the patient has a complement-associated disorder.

59. The method of any one of claims 56 to 58, wherein administration of the inhibitor reduces the occurrence or severity of TMA in the brain of the patient.

60. The method of any one of claims 56 to 59, wherein administration of the inhibitor reduces the occurrence or severity of TMA in the kidney.

61. The method of any one of claims 56 to 60, wherein administration of the inhibitor to the patient promotes the resolution of pre-existing TMA in the patient.

62. A method for ameliorating one or more symptoms associated with a complement-associated disorder, the method comprising administering to a patient in need thereof an inhibitor of human complement in an amount effective to ameliorate one or more symptoms associated a complement-associated disorder, wherein the symptoms are ameliorated within less than 14 days after administering the inhibitor and with the proviso that the complement-associated disorder is not paroxysmal nocturnal hemoglobinuria.

63. The method of claim 62, wherein the one or more symptoms are ameliorated in less than 10 days after administering the inhibitor.

64. The method of claim 62 or 63, wherein the one or more symptoms are ameliorated in less than 5 days after administering the inhibitor.

65. The method of any one of claims 62 to 64, wherein the one or more symptoms are ameliorated in less than 2 days after administering the inhibitor.

66. The method of any one of claims 62 to 65, wherein the one or more symptoms are ameliorated in less than 1 day after administering the inhibitor.

67. The method of any one of claims 62 to 66, wherein the one or more symptoms are ameliorated in less than 12 hours after administering the inhibitor.

68. The method of any one of claims 62 to 67, wherein the one or more symptoms are selected from the group consisting of proteinuria, elevated LDH levels, hypertension, reduced platelet counts, and reduced urine output.

69. The method of any one of claims 62 to 68, wherein at least one of the one or more symptoms is ameliorated to within 40% of normal.

70. The method of any one of claims 62 to 69, wherein at least one of the one or more symptoms is ameliorated to within 20% of normal.

71. The method of any one of claims 62 to 70, wherein at least one of the one or more symptoms is completely alleviated in the patient.

72. A method for treating a complement-associated disorder, the method comprising administering to a patient afflicted with a complement-associated disorder an inhibitor of human complement in an amount effective to treat the complement-associated disorder, wherein the inhibitor is administered to the patient even after one or more symptoms of the disorder have been ameliorated and with the proviso that the complement-associated disorder is not paroxysmal nocturnal hemoglobinuria.

73. The method of claim 72, wherein the inhibitor is administered to the patient even after one or more symptoms have been completely ameliorated.

74. The method of claim 72 or 73, wherein the inhibitor is administered to the patient even after the patient has entered clinical remission.

75. The method of any one of claims 62 to 74, wherein the inhibitor is administered to the patient for at least two months after one or more symptoms of the disorder have been ameliorated.

76. The method of any one of claims 62 to 75, wherein the inhibitor is administered to the patient for at least six months after one of more symptoms of the disorder have been ameliorated.

77. The method of any one of claims 62 to 76, wherein the inhibitor is administered to the patient for at least one year after one or more symptoms of the disorder have been ameliorated.

78. The method of any one of claims 62 to 77, wherein the inhibitor is administered to the patient for at least two years after one or more symptoms of the disorder have been ameliorated.

79. The method of any one of claims 62 to 78, wherein the inhibitor is chronically administered to the patient.

80. The method of any one of claims 1 or 4 to 79, wherein the complement-associated disorder is selected from the group consisting of membranoproliferative glomerulonephritis, Degos disease, atypical hemolytic uremic syndrome, antibody-mediated rejection, HELLP syndrome, and catastrophic antiphospholipid syndrome.

81. A method for treating aHUS, the method comprising administering to a patient in need thereof an inhibitor of complement component C5a in an amount effective to treat aHUS in the patient.

82. The method of claim 81, wherein the inhibitor is: (i) an antibody that binds to C5a; or (ii) an antigen-binding fragment of the antibody.

83. The method of claim 81 or 82, wherein the antibody binds to a human C5a comprising the amino acid sequence depicted in SEQ ID NO:12.

84. The method of any one of claims 81 to 83, wherein the antibody is a monoclonal antibody.

85. The method of any one of claims 81 or 84, wherein the antibody is a single-chain antibody.

86. The method of any one of claims 81 or 85, wherein the antibody is a humanized antibody.

87. The method of any one of claims 81 to 86, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of a recombinant antibody, a diabody, a chimerized or chimeric antibody, a deimmunized human antibody, a fully human antibody, a single chain antibody, an Fv fragment, an Fd fragment, an Fab fragment, an Fab' fragment, and an $F(ab')_2$ fragment.

88. The method of any one of claims 81 to 87, wherein the inhibitor ameliorates one or both of hypertension and vasoconstriction in the patient.

89. The method of claim 88, wherein the hypertension is ameliorated within less than 7 days following administration of the inhibitor.

90. The method of any one of claims 81 to 89, wherein the inhibitor is chronically administered to the patient.

91. An article of manufacture comprising:

a container comprising a label; and

a composition comprising an inhibitor of human complement component C5, wherein the label indicates that the composition is to be administered to a human having, suspected of having, or at risk for developing, a complement-associated disorder with the proviso that the complement-associated disorder is not paroxysmal nocturnal hemoglobinuria.

92. The article of manufacture of claim 91, wherein the inhibitor is an antibody or antigen-binding fragment thereof that binds to a human complement component C5 protein.

93. The article of manufacture of claim 91 or 92, further comprising one or more additional active agents.

AMENDED CLAIMS
received by the International Bureau on 09 April 2010 (09.04.2010)

What is claimed is:

1. A method for treating a complement-associated disorder, the method comprising chronically administering to a patient in need thereof a complement inhibitor in an amount and with a frequency that are effective to substantially inhibit systemic complement activity in the patient, wherein the patient has, is suspected of having, or is at risk for developing a complement-associated disorder and with the proviso that the complement-associated disorder is not paroxysmal nocturnal hemoglobinuria.

2. A method for transplanting an allogeneic organ or tissue, the method comprising transplanting an organ or tissue into a patient in need thereof, wherein prior to and following the transplanting a complement inhibitor is administered to the patient in an amount and with a frequency effective to substantially inhibit systemic complement activity in the patient.

3. The method of claim 2, wherein the organ or tissue is selected from the group consisting of a liver, a kidney, a heart, a lung, skin, eye, bone marrow, and vascular tissue.

4. The method of claim 2 or 3, wherein the patient has, is suspected of having, or is at risk for developing a complement-associated disorder.

5. The method of any one of claims 1 to 4, wherein the complement inhibitor is chronically administered to a patient in an amount and with a frequency that are effective to reduce serum hemolytic activity to less than or equal to 20% and to maintain the serum hemolytic activity at less than or equal to 20%.

6. The method of any one of claims 1 to 5, wherein the complement inhibitor is chronically administered to a patient in an amount and with a frequency that are effective to maintain a serum LDH level that is less than or equal to 500 IU/L.

7. The method of any one of claims 1 to 6, wherein the complement inhibitor is selected from the group consisting of a polypeptide, a polypeptide analog, a nucleic acid, a nucleic acid analog, and a small molecule.

8. The method of any one of claims 1 to 7, wherein the complement inhibitor is selected from the group consisting of soluble CR1, LEX-CR1, MCP, DAF, CD59, Factor H, cobra venom factor, FUT-175, complestatin, and K76 COOH.

9. The method of any one of claims 1 to 7, wherein the complement inhibitor inhibits the expression of a human complement component protein.

10. The method of any one of claims 1 to 7, wherein the complement inhibitor inhibits the cleavage of human complement component C5, C4, C3, or C2.

11. The method of claim 10, wherein the complement inhibitor inhibits the cleavage of complement component C5 into fragments C5a and C5b.

12. The method of claim 10 or 11, wherein the complement inhibitor is an antibody or antigen-binding fragment thereof that binds to a human complement component protein.

13. The method of claim 12, wherein the antibody or antigen-binding fragment thereof binds to a human complement component C5 protein.

14. The method of claim 13, wherein the antibody binds to the alpha chain of the complement component C5 protein.

15. The method of claim 13 or 14, wherein the antibody binds to the alpha chain of human complement component C5, and wherein the antibody (i) inhibits complement activation in a human body fluid, (ii) inhibits the binding of purified human complement

component C5 to either human complement component C3b or human complement component C4b, and (iii) does not bind to the human complement activation product free C5a.

16. The method of claim 13, wherein the antibody binds to the human complement component C5 protein having the amino acid sequence depicted in any one of SEQ ID NOs:1-26.

17. The method of claim 13, wherein the antibody binds to an isolated oligopeptide comprising an amino acid sequence corresponding to amino acid position 8 through amino acid position 12 of SEQ ID NO:5.

18. The method of claim 12 or 13, wherein the polypeptide comprises an antibody that binds to complement component C5 fragment C5b.

19. The method of any one of claims 12 to 18, wherein the antibody is a monoclonal antibody.

20. The method of any one of claims 12 to 19, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of a humanized antibody, a recombinant antibody, a diabody, a chimerized or chimeric antibody, a deimmunized human antibody, a fully human antibody, a single chain antibody, an Fv fragment, an Fd fragment, an Fab fragment, an Fab' fragment, and an $F(ab')_2$ fragment.

21. The method of claim 12 or 13, wherein the antibody is eculizumab.

22. The method of any one of claims 12, 13, or 21, wherein the antibody is administered to the patient in an amount and with a frequency to maintain a concentration of at least 0.7 anti-C5 antibody molecules per every C5 molecule in the patient's blood.

23. The method of any one of claims 12, 13, or 21, wherein the antibody is administered to the patient in an amount and with a frequency to maintain at least 50 μg of the antibody per milliliter of the patient's blood.

24. The method of claim 23, wherein the antibody is administered to the patient in an amount and with a frequency effective to maintain a concentration of at least 100 μg of the antibody per milliliter of the patient's blood.

25. The method of any one of claims 1 to 24, wherein the body weight of the patient is greater than or equal to 40 kg.

26. The method of any one of claims 12, 13, or 21 to 25, wherein the antibody is administered to the patient for at least 7 weeks under the following schedule:

at least 800 mg of the antibody, once per week for four consecutive weeks;

at least 800 mg of the antibody once during the fifth week; and

at least 800 mg of the antibody bi-weekly thereafter.

27. The method of claim 26, wherein the antibody is administered to the patient for at least 7 weeks under the following schedule:

at least 900 mg of the antibody, once per week for four consecutive weeks;

at least 1200 mg of the antibody once during the fifth week; and

at least 1200 mg of the antibody bi-weekly thereafter.

28. The method of any one of claims 1 to 24, wherein the body weight of the patient is less than 40 kg but greater than or equal to 30 kg.

29. The method of claim 28, wherein the antibody is chronically administered to the patient for at least 5 weeks under the following schedule:

at least 500 mg of the antibody, once per week for two consecutive weeks;

at least 700 mg of the antibody once during the third week; and

at least 700 mg of the antibody bi-weekly thereafter.

30. The method of claim 29, wherein the antibody is chronically administered to the patient for at least 5 weeks under the following schedule:

at least 600 mg of the antibody, once per week for two consecutive weeks;

at least 900 mg of the antibody once during the third week; and

at least 900 mg of the antibody bi-weekly thereafter.

31. The method of any one of claims 1 to 24, wherein the body weight of the patient is less than 30 kg, but is greater than or equal to 20 kg.

32. The method of claim 31, wherein the antibody is chronically administered to the patient for at least 5 weeks under the following schedule:

at least 500 mg of the antibody, once per week for two consecutive weeks;

at least 500 mg of the antibody once during the third week; and

at least 500 mg of the antibody bi-weekly thereafter.

33. The method of claim 32, wherein the antibody is chronically administered to the patient for at least 5 weeks under the following schedule:

at least 600 mg of the antibody, once per week for two consecutive weeks;

at least 600 mg of the antibody once during the third week; and

at least 600 mg of the antibody bi-weekly thereafter.

34. The method of any one of claims 1 to 24, wherein the body weight of the patient is less than 20 kg, but is greater than or equal to 10 kg.

35. The method of claim 34, wherein the antibody is chronically administered to the patient for at least 4 weeks under the following schedule:

at least 500 mg of the antibody once a week for one week;

at least 200 mg of the antibody once during the second week; and

at least 200 mg of the antibody bi-weekly thereafter.

36. The method of claim 36, wherein the antibody is chronically administered to the patient for at least 4 weeks under the following schedule:

at least 600 mg of the antibody once a week for one week;

at least 300 mg of the antibody once during the second week; and

at least 300 mg of the antibody bi-weekly thereafter.

37. The method of any one of claims 1 to 24, wherein the body weight of the patient is less than 10 kg, but is greater than or equal to 5 kg.

38. The method of claim 37, wherein the antibody is chronically administered to the patient for at least 5 weeks under the following schedule:

at least 200 mg of the antibody, once per week for one week;

at least 200 mg of the antibody once during the second week; and

at least 200 mg of the antibody once every three weeks thereafter.

39. The method of claim 38, wherein the antibody is chronically administered to the patient for at least 5 weeks under the following schedule:

at least 300 mg of the antibody, once per week for one week;

at least 300 mg of the antibody once during the second week; and

at least 300 mg of the antibody every three weeks thereafter.

40. The method of any one of claims 2 to 24, wherein the complement inhibitor is administered to the patient less than 24 hours prior to the transplanting.

41. The method of any one of claims 2 to 24 or 40, wherein the complement inhibitor is administered to the patient less than 12 hours prior to transplanting.

42. The method of any one of claims 2 to 24, 40, or 41, wherein the complement inhibitor is administered to the patient less than 24 hours following the transplanting of the organ or tissue.

43. The method of any one of claims 2 to 24, 40, 41, or 42, wherein the complement inhibitor is administered to the patient less than 12 hours following the transplanting of the organ or tissue.

44. The method of claim 2 to 4 or 40 to 43, wherein the complement inhibitor is a whole antibody that binds to complement component C5 and, prior to the transplanting, at least 900 mg of the antibody is administered to the patient.

45. The method of claim 44, wherein, prior to the transplanting, at least 1,200 mg of the antibody is administered to the patient.

46. The method of any one of claims 2 to 4 or 40 to 45, wherein the complement inhibitor is a whole antibody that binds to complement component C5 and, following the transplanting, at least 800 mg of the antibody is administered to the patient.

47. The method of claim 46, wherein following the transplanting, at least 900 mg of the antibody is administered to the patient.

48. The method of claim 46 or 47, wherein following the transplanting, the anti-C5 antibody is administered to the patient for at least 7 weeks under the following schedule:

at least 800 mg of the anti-C5 antibody less than 24 hours after transplanting the organ or tissue;

at least 800 mg of the anti-C5 antibody once per week for four weeks;

at least 800 mg of the anti-C5 antibody once during the fifth week; and

at least 800 mg of the anti-C5 antibody bi-weekly thereafter.

49. The method of any one of claims 46, 47, or 48, wherein following the transplanting, the anti-C5 antibody is administered to the patient for at least 7 weeks under the following schedule:

at least 1200 mg of the anti-C5 antibody less than 24 hours after transplanting the organ or tissue;

at least 900 mg of the anti-C5 antibody once per week for four weeks;

at least 1200 mg of the anti-C5 antibody once during the fifth week; and

at least 1200 mg of the anti-C5 antibody bi-weekly thereafter.

50. The method of any one of claims 26, 27, 29, 30, 32, 33, 35, 36, 38, 39, 48, or 49, wherein the antibody is administered to the patient for at least 21 weeks under the schedule.

51. The method of any one of claims 26, 27, 29, 30, 32, 33, 35, 36, 38, 39, 48, 49, or 50, wherein the antibody is administered to the patient for at least one year under the schedule.

52. The method of any one of claims 26, 27, 29, 30, 32, 33, 35, 36, 38, 39, or 48 to 51, wherein the inhibitor is administered to the patient for the remainder of the patient's life.

53. The method of any one of claims 1 to 25, 28, 31, 34, 37, or 40 to 47, wherein the inhibitor is chronically administered to the patient at least once per week.

54. The method of any one of claims 1 to 25, 28, 31, 34, 37, or 40 to 47, wherein the inhibitor is chronically administered to the patient at least once every two weeks.

55. The method of any one of claims 1 to 54, wherein the inhibitor is chronically administered to the patient for the remainder of the patient's life.

56. A method for treating thrombotic microangiopathy (TMA) in a patient who has, is suspected of having, or at risk of developing TMA, the method comprising administering to a patient in need thereof an inhibitor of human complement to thereby treat TMA in the patient.

57. A method for reducing the occurrence or severity of thrombotic microangiopathy (TMA) in a patient who has, is suspected of having, or at risk of developing TMA, the method comprising administering to a patient in need thereof an inhibitor of human complement to thereby reduce the occurrence or severity of TMA in the patient.

58. The method of claim 56 or 57, wherein the patient has a complement-associated disorder.

59. The method of any one of claims 56 to 58, wherein administration of the inhibitor reduces the occurrence or severity of TMA in the brain of the patient.

60. The method of any one of claims 56 to 59, wherein administration of the inhibitor reduces the occurrence or severity of TMA in the kidney.

61. The method of any one of claims 56 to 60, wherein administration of the inhibitor to the patient promotes the resolution of pre-existing TMA in the patient.

62. A method for ameliorating one or more symptoms associated with a complement-associated disorder, the method comprising administering to a patient in need thereof an inhibitor of human complement in an amount effective to ameliorate one or more symptoms associated a complement-associated disorder, wherein the symptoms are ameliorated within less than 14 days after administering the inhibitor and with the proviso that the complement-associated disorder is not paroxysmal nocturnal hemoglobinuria.

63. The method of claim 62, wherein the one or more symptoms are ameliorated in less than 10 days after administering the inhibitor.

64. The method of claim 62 or 63, wherein the one or more symptoms are ameliorated in less than 5 days after administering the inhibitor.

65. The method of any one of claims 62 to 64, wherein the one or more symptoms are ameliorated in less than 2 days after administering the inhibitor.

66. The method of any one of claims 62 to 65, wherein the one or more symptoms are ameliorated in less than 1 day after administering the inhibitor.

67. The method of any one of claims 62 to 66, wherein the one or more symptoms are ameliorated in less than 12 hours after administering the inhibitor.

68. The method of any one of claims 62 to 67, wherein the one or more symptoms are selected from the group consisting of proteinuria, elevated LDH levels, hypertension, reduced platelet counts, and reduced urine output.

69. The method of any one of claims 62 to 68, wherein at least one of the one or more symptoms is ameliorated to within 40% of normal.

70. The method of any one of claims 62 to 69, wherein at least one of the one or more symptoms is ameliorated to within 20% of normal.

71. The method of any one of claims 62 to 70, wherein at least one of the one or more symptoms is completely alleviated in the patient.

72. A method for treating a complement-associated disorder, the method comprising administering to a patient afflicted with a complement-associated disorder an inhibitor of human complement in an amount effective to treat the complement-associated disorder, wherein the inhibitor is administered to the patient even after one or more symptoms of the disorder have been ameliorated and with the proviso that the complement-associated disorder is not paroxysmal nocturnal hemoglobinuria.

73. The method of claim 72, wherein the inhibitor is administered to the patient even after one or more symptoms have been completely ameliorated.

74. The method of claim 72 or 73, wherein the inhibitor is administered to the patient even after the patient has entered clinical remission.

75. The method of any one of claims 62 to 74, wherein the inhibitor is administered to the patient for at least two months after one or more symptoms of the disorder have been ameliorated.

76. The method of any one of claims 62 to 75, wherein the inhibitor is administered to the patient for at least six months after one of more symptoms of the disorder have been ameliorated.

77. The method of any one of claims 62 to 76, wherein the inhibitor is administered to the patient for at least one year after one or more symptoms of the disorder have been ameliorated.

78. The method of any one of claims 62 to 77, wherein the inhibitor is administered to the patient for at least two years after one or more symptoms of the disorder have been ameliorated.

79. The method of any one of claims 62 to 78, wherein the inhibitor is chronically administered to the patient.

80. The method of any one of claims 1 or 4 to 79, wherein the complement-associated disorder is selected from the group consisting of membranoproliferative glomerulonephritis, atypical hemolytic uremic syndrome, antibody-mediated rejection, HELLP syndrome, and catastrophic antiphospholipid syndrome.

81. A method for treating aHUS, the method comprising administering to a patient in need thereof an inhibitor of complement component C5a in an amount effective to treat aHUS in the patient.

82. The method of claim 81, wherein the inhibitor is: (i) an antibody that binds to C5a; or (ii) an antigen-binding fragment of the antibody.

83. The method of claim 81 or 82, wherein the antibody binds to a human C5a comprising the amino acid sequence depicted in SEQ ID NO:12.

84. The method of any one of claims 81 to 83, wherein the antibody is a monoclonal antibody.

85. The method of any one of claims 81 or 84, wherein the antibody is a single-chain antibody.

86. The method of any one of claims 81 or 85, wherein the antibody is a humanized antibody.

87. The method of any one of claims 81 to 86, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of a recombinant antibody, a diabody, a chimerized or chimeric antibody, a deimmunized human antibody, a fully human antibody, a single chain antibody, an Fv fragment, an Fd fragment, an Fab fragment, an Fab' fragment, and an $F(ab')_2$ fragment.

88. The method of any one of claims 81 to 87, wherein the inhibitor ameliorates one or both of hypertension and vasoconstriction in the patient.

89. The method of claim 88, wherein the hypertension is ameliorated within less than 7 days following administration of the inhibitor.

90. The method of any one of claims 81 to 89, wherein the inhibitor is chronically administered to the patient.

91. An article of manufacture comprising:

a container comprising a label; and

a composition comprising an inhibitor of human complement component C5, wherein the label indicates that the composition is to be administered to a human having, suspected of having, or at risk for developing, a complement-associated disorder with the proviso that the complement-associated disorder is not paroxysmal nocturnal hemoglobinuria.

92. The article of manufacture of claim 91, wherein the inhibitor is an antibody or antigen-binding fragment thereof that binds to a human complement component C5 protein.

93. The article of manufacture of claim 91 or 92, further comprising one or more additional active agents.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2009/063929

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 39/00 (2006.01) ***A61K 38/00*** (2006.01) ***C07K 16/24*** (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPODOC, MEDLINE, WPI
Keywords: Complement, C5, Antibody, Inhibition, Eculizumab, Transplant, Thrombotic Microangiopathy, Haemolytic Uremic Syndrome and related terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/153962 A2 (THE TRUSTEES OF THE UNIVERSITY OF PENNYLVANIA) 18 December 2008 *Abstract, Pages 10-17, Examples*	1, 2, 4-7, 9-55, 62-80, 91-93
X	WO 2007/038995 A1 (ZLB BEHRING GMBH) 12 April 2007 *Abstract, Pages 1-8*	1, 5-10, 25, 28, 31, 34, 37, 56-80
X	WO 2005/110481 A2 (ALEXION PHARMACEUTICALS INC.) 24 November 2005 *Abstract, Pages 16-16*	1-7, 9-55, 62-80, 91-93

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 January 2010	- 9 FEB 2010
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999	Authorized officer **JOSE PEREGRINA** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6283 2687

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2009/063929

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6169068 B1 (LEVIN *et al.*) 2 January 2001 *Abstract, Tables, Column 19-22*	1-10, 25, 28, 31, 34, 37, 53-55, 62-80
X	WO 1991/015221 A1 (IMUTRAN LTD.) 17 October 1991 *Abstract, Pages 1-14, Examples*	1-10, 12, 19, 20, 25-39, 40-43, 53-55, 62-80
X	PRATT J R *et al*, "Effects of Complement Inhibition with Soluble Complement Receptor 1 on Vascular Injury and Inflammation during Renal Allograft Rejection in the Rat," American Journal of Pathology, Vol. 149, No. 6, December 1996, Pages 2055-2066 *The whole document*	1-10, 25, 28, 31, 34, 37, 53-55, 62-80
X	NANGAKU M *et al*, "CD59 Protects Glomerular Endothelial Cells from Immune-Mediated Thrombotic Microangiopathy in Rats," Journal of the American Society of Nephrology, Vol. 9, No. 4, April 1998, Pages 590-597 *The whole document*	1, 8-11, 55-80

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2009/063929

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2008/153962	NONE					
WO	2007/038995	AU	2006299293	CA	2627264	EP	1928483
		KR	20080048034	US	2009118163		
WO	2005/110481	AU	2005244012	CA	2566716	EP	1755674
		NZ	551308	US	2009028850		
US	6169068	AU	62372/94	CA	2155933	EP	0682526
		WO	1994/17822				
WO	1991/015221	AU	75555/91	CN	1056240	CS	9100988
		EP	0527762	HK	48597	IE	911153
		IL	97788	IN	173149	NZ	237739
		PT	97277	SG	47465	US	2001018051
		ZA	9102467				

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX