The present disclosure relates to bispecific antibodies that can bind to two or more different epitopes. For example, the bispecific antibodies described herein can bind to two or more different proteins, wherein at least two of the proteins are selected from C5a, C5b, a cellular receptor for C5a (e.g., C5aR) or C5L2, the C5b-9 complex, and a component or intermediate of terminal complement such as C5b-6, C5b-7, or C5b-8. The bispecific antibodies described herein are useful for, e.g., inhibiting terminal complement (e.g., the assembly and/or activity of the C5b-9 complex) and/or C5a anaphylatoxin-mediated inflammation (e.g., C5a-mediated chemotaxis of inflammatory immune cells). Accordingly, the bispecific antibodies can be used in methods for treating a variety of complement pathway-associated disorders.
BISPECIFIC ANTIBODIES THAT BIND TO COMPLEMENT PROTEINS

Cross-Reference to Related Applications

This application claims the benefit of U.S. provisional patent application serial nos.: 61/219,644, filed on June 23, 2009, and 61/228,001, filed on July 23, 2009, the disclosures of each of which are incorporated herein by reference in their entirety.

Sequence Listing

The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 22, 2010, is named ALXN149W01.txt, and is 25,951 bytes in size.

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 fraction of blood. This process, also known as "tickover," occurs through the spontaneous cleavage of a thioester bond in C3 to form C3i or C3(H₂O). 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(H₂O) 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(H₂O)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)₂Bb - 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 AcadSci  USA 77: 559-562).

The CP C3 convertase is formed upon interaction of complement component Cl, which is a complex of Clq, C1r, and C1s, with an antibody that is bound to a target antigen (e.g., a microbial antigen). The binding of the Clq portion of Cl to the antibody-antigen complex causes a conformational change in Cl that activates C1r.

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Active CIr then cleaves the C1-associated CIs to thereby generate an active serine protease. Active CIs 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). CIs 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 into C5a and C5b. Cleavage of C5 releases C5a, a potent anaphylatoxin and chemotactic factor, and C5b, which allows for the formation of the lytic terminal complement complex, C5b-9. C5b combines with C6, C7, and C8 to form the C5b-8 complex at the surface of the target cell. 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.

While a properly functioning complement system provides a robust defense against infecting microbes, inappropriate regulation or activation of the complement pathways has been implicated in the pathogenesis of a variety of disorders including, e.g., rheumatoid arthritis (RA); lupus nephritis; asthma; ischemia-reperfusion injury; atypical hemolytic uremic syndrome (aHUS); dense deposit disease (DDD); paroxysmal nocturnal hemoglobinuria (PNH); macular degeneration (e.g., age-related macular degeneration (AMD)); hemolysis, elevated liver enzymes, and low platelets

Summary

The present disclosure relates to bispecific antibodies that bind to human complement component proteins. A bispecific antibody described herein can bind to two or more (e.g., two, three, four, five, or six or more) different epitopes. For example, a bispecific antibody described herein can bind to two or more (e.g., two, three, four, five, or six or more) different proteins, wherein at least two of the proteins are selected from C5a, C5b, a cellular receptor for C5a (e.g., C5aR1 or C5L2), and a component or an intermediate of the terminal complement complex such as C5b-6, C5b-7, C5b-8, or C5b-9. For example, in some embodiments, a bispecific antibody can bind to two different proteins selected from the group consisting of C5a, C5aR, C5b, C5L2, C5b-6, C5b-7, C5b-8, and C5b-9. The bispecific antibodies are useful for inhibiting terminal complement (e.g., the assembly and/or activity of the C5b-9 TCC) and/or C5a anaphylatoxin-mediated inflammation. Accordingly, the bispecific antibodies can be used in methods for treating a variety of complement pathway-associated disorders such as, but not limited to, atypical hemolytic uremic syndrome (aHUS); thrombotic thrombocytopenic purpura (TTP); dense deposit disease (DDD); rheumatoid arthritis (RA); hemolysis, elevated liver enzymes, and low platelets (HELLP); age-related macular degeneration (AMD); myasthenia gravis (MG), cold
agglutinin-disease (CAD), dermatomyositis, Graves’ disease, Hashimoto’s thyroiditis, type I diabetes, psoriasis, pemphigus, autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), or any other complement-associated disorder described herein and/or known in the art.

The bispecific antibodies described herein feature a number of advantages, e.g., advantages over antibodies that bind to, and inhibit cleavage of, full-length or mature C5. Like such anti-C5 antibodies, the bispecific antibodies described herein are capable of inhibiting the downstream effects of one or both arms of C5 activation. That is, in some embodiments, the bispecific antibodies described herein can inhibit the C5a-mediated inflammatory response and the C5b (MAC)-dependent cell lysis that results from cleavage of C5. However, as the concentration of C5 in human plasma is approximately 0.37 µM (Rawal and Pangburn (2001) J Immunol 166(4):2635-2642), the use of high concentrations and/or frequent administration of anti-C5 antibodies is often necessary to effectively inhibit C5 in a human. Unlike C5, fragments C5a and C5b are present in blood at much lower concentrations and are often restricted to specific areas of local complement activation such as, e.g., the lungs in asthma patients, the joints of RA patients, or the drusen in the eyes of patients with AMD.

In addition, the disclosure sets forth in the working examples experimental data evidencing that while anti-C5 antibodies are highly effective at inhibiting complement in vitro and in vivo (see, e.g., Hillmen et al. (2004) N Engl J Med 350(6):552), the antibodies are particularly susceptible to target-mediated clearance because of the high concentration of C5 in blood. This discovery indicates that bispecific antibodies (e.g., bispecific antibodies that bind to C5a and a component of the MAC such as C5b) are very likely to have a longer half-life, as compared to anti-C5 antibodies, in blood due to a reduced contribution of antigen-mediated antibody clearance. As described above, fragments C5a and C5b are each present at a much lower concentration than C5 and are often produced at specific areas of complement activation. Thus, in view of their longer half-life, the bispecific antibodies described herein can be administered to a human at a much lower dose and/or less frequently than an anti-C5 antibody and effectively provide the same or greater inhibition of C5 in a human. The ability to administer a lower dose of the bispecific antibody, as compared to dose of an anti-C5 antibody, also allows for additional delivery routes
such as, e.g., subcutaneous administration, intramuscular administration, intrapulmonary delivery, and administration via the use of biologically degradable microspheres.

The bispecific antibodies described herein also provide a number of advantages over the use of a combination of two different antibodies. For example, a bispecific antibody that binds to C5a and C5b, being one molecule, requires only one process development as compared to two different processes required to produce a separate anti-C5a antibody and an anti-C5b antibody. Moreover, administration of a cocktail of two different antibodies (e.g., an anti-C5a antibody and an anti-C5b antibody) would also likely require a significantly more complex clinical evaluation. For example, safety studies and pharmacokinetic (PK) and pharmacodynamic (PD) parameters for each separate antibody would need to be evaluated to ensure not only equivalence between the two antibodies, but also that neutralization of both of the antibodies' intended targets (e.g., C5a and C5b) is maintained over time. In addition, targeting only the active C5 fragments (C5a and C5b) or their receptors means that the clearance of the bispecific antibody is unlikely to be significantly influenced by the normal clearance or turnover of a native, highly abundant plasma C5 protein.

In one aspect, the disclosure features a bispecific antibody that binds to at least two of: C5a, C5b, a cellular receptor for C5a (e.g., C5aR1 or C5L2), a component of or an intermediate of the TCC; or the TCC (C5b-9) itself. The component of the TCC can be, e.g., C5b, C6, C7, C8, C9, or a biologically-active fragment thereof. The intermediate of the TCC can be, e.g., C5b-6, C5b-7, or C5b-8. In some embodiments, the antibody binds to C5b-9.

In another aspect, the disclosure features a bispecific antibody that binds to:

(a) C5a and C5aR1; (b) C5a and C5b; (c) C5b and C5aR1; (d) C5a and C5L2; (e) C5b and C5L2; (f) C5aR1 and C5L2; (g) C5b-6 and C5a; (h) C5b-6 and C5b; (i) C5b-6 and C5aR1; or (j) C5b-6 and C5L2.

In another aspect, the disclosure features a bispecific antibody containing at least two (or consisting of two) different antigen combining sites, wherein at least one antigen combining site binds to C5a and at least one antigen combining site binds to C5b or C5aR. The disclosure also features an antibody containing at least two (or consisting of two) different antigen combining sites, wherein at least one antigen combining site binds to C5b and at least one antigen combining site binds to C5aR.
The disclosure further features an antibody containing at least two different antigen combining sites, wherein (i) at least one antigen combining site binds to C5a or C5b; and (ii) at least one antigen combining site binds to a cellular receptor for C5a such as C5aR1 or C5L2. The disclosure also features an antibody containing at least two (or consisting of two) different antigen combining sites, wherein (i) at least one antigen combining site binds to C5a, C5b, or a cellular receptor for C5a; and (ii) at least one antigen combining site binds to C5b-6. In some embodiments, any of these antibodies can contain two or more than two (e.g., three, four, five, six, seven, eight, nine, or 10 or more) antigen combining sites.

In another aspect, the disclosure features a bispecific antibody having binding specificity for at least two of C5a, C5aR1, C5b, C5L2, C6, Cl, C8, C9, C5b-6, C5b-7, C5b-8, and C5b-9. In some embodiments, the antibody has binding specificity for at least two of C5a, C5b, C5aR1, and C5b-6.

In some embodiments of any of the antibodies described herein, the antibody does not bind to full-length or mature C5. In some embodiments, the antibody does not bind to uncomplexed C5b, C6, C7, C8, or C9.

In yet another aspect, the disclosure features a bispecific antibody containing or consisting of: (i) a first antigen combining site that binds to C5a; and (ii) a second antigen combining site that binds to a cellular receptor for C5a. The first antigen combining site can bind to desargerrated C5a. The first antigen combining site can bind to a mammalian C5a (e.g., human C5a). The first antigen combining site can bind to a human C5a protein containing or consisting of an amino acid sequence that is at least 70 (e.g., at least 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 even 100) % identical to the amino acid sequence depicted in SEQ ID NO: 1. The first antigen combining site can bind to a fragment of a human C5a protein containing or consisting of the amino acid sequence depicted in any one of SEQ ID NOs: 2-14. The first antigen combining site can bind to an epitope comprising at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more) consecutive amino acids depicted in any one of SEQ ID NOs: 1-14. In some embodiments, the cellular receptor for C5a is C5aR1. C5aR1 can be a mammalian (e.g., a human) form of C5aR1. The second antigen combining site can bind to a human C5aR1 protein comprising an amino acid sequence that is at least 70 (e.g., at least 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 even 100) % identical to the amino acid sequence depicted in SEQ ID NO: 17. The second antigen combining site can bind to a fragment of a human C5aRI protein comprising the amino acid sequence depicted in any one of SEQ ID NOs: 18-22. The second antigen combining site can bind to an epitope comprising at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more) consecutive amino acids depicted in any one of SEQ ID NOs: 17-22. In some embodiments, the cellular receptor for C5a is C5L2. C5L2 can be a mammalian (e.g., a human) form of C5L2. The second antigen combining site can bind to a human C5L2 protein comprising an amino acid sequence that is at least 70 (e.g., at least 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 even 100) % identical to the amino acid sequence depicted in SEQ ID NO: 23. In some embodiments, the second antigen combining site can bind to a fragment of a human C5L2 at an epitope containing at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more) consecutive amino acids of SEQ ID NO: 23. The second antigen combining site can bind to a fragment of a human C5L2 protein comprising the amino acid sequence depicted in any one of SEQ ID NOs: 24-27. The second antigen combining site can bind to an epitope comprising at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more) consecutive amino acids depicted in any one of SEQ ID NOs: 24-27. In some embodiments, the antibody does not bind to full-length or mature C5.

In another aspect, the disclosure features a bispecific antibody containing or consisting of: (i) a first antigen combining site that binds to C5a; and (ii) a second antigen combining site that binds to C5b. The first antigen combining site can bind to desarginated C5a. The first antigen combining site can bind to a mammalian C5a (e.g., human C5a). The first antigen combining site can bind to a human C5a protein containing or consisting of an amino acid sequence that is at least 70 (e.g., at least 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 even 100) % identical to the amino acid sequence depicted in SEQ ID NO: 1. The first antigen combining site can bind to a fragment of a human C5a protein containing or consisting of the amino acid sequence depicted in any one of SEQ ID NOs: 2-14. The first antigen combining site can bind to an epitope
comprising at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more) consecutive amino acids depicted in any one of SEQ ID NOs: 1-14. In some embodiments, the second antigen combining site binds to a mammalian (e.g., a human) form of C5b. The human C5b can contain or consist of an amino acid sequence that is at least 70 (e.g., at least 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 even 100) % identical to the amino acid sequence depicted in SEQ ID NO: 15 or 16. The second antigen combining site can bind to an epitope that contains, or consists of, at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more) consecutive amino acids depicted in SEQ ID NO: 15 or 16. In some embodiments, the antibody does not bind to full-length or mature C5.

In yet another aspect, the disclosure features a bispecific antibody that contains, or consists of: (i) a first antigen combining site that binds to C5b; and (ii) a second antigen combining site that binds to a cellular receptor for C5a. In some embodiments, the second antigen combining site binds to a mammalian (e.g., a human) form of C5b. The human C5b can contain or consist of an amino acid sequence that is at least 70 (e.g., at least 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 even 100) % identical to the amino acid sequence depicted in SEQ ID NO: 15 or 16. The first antigen combining site can bind to an epitope that contains, or consists of, at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more) consecutive amino acids depicted in SEQ ID NO: 15 or 16. In some embodiments, the antibody does not bind to full-length or mature C5. In some embodiments, the cellular receptor for C5a is C5aRI. C5aRI can be a mammalian (e.g., a human) form of C5aRI. The second antigen combining site can bind to a human C5aRI protein comprising an amino acid sequence that is at least 70 (e.g., at least 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 even 100) % identical to the amino acid sequence depicted in SEQ ID NO: 17. The second antigen combining site can bind to a fragment of a human C5aRI protein comprising the amino acid sequence depicted in any one of SEQ ID NOs: 18-22. The second antigen combining site can bind to an epitope comprising at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more) consecutive amino acids depicted in any one
of SEQ ID NOs: 17-22. In some embodiments, the cellular receptor for C5a is C5L2. C5L2 can be a mammalian (e.g., a human) form of C5L2. The second antigen combining site can bind to a human C5L2 protein comprising an amino acid sequence that is at least 70 (e.g., at least 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 even 100) % identical to the amino acid sequence depicted in SEQ ID NO:23. In some embodiments, the second antigen combining site can bind to a fragment of a human C5L2 at an epitope containing at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more) consecutive amino acids of SEQ ID NO:23. The second antigen combining site can bind to a fragment of a human C5L2 protein comprising the amino acid sequence depicted in any one of SEQ ID NOs:24-27. The second antigen combining site can bind to an epitope comprising at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 or more) consecutive amino acids depicted in any one of SEQ ID NOs:24-27.

In another aspect, the disclosure provides a bispecific antibody that contains, or consists of: (i) a first antigen combining site that binds to C5a, C5b, or a cellular receptor for C5a; and (ii) a second antigen combining site that binds to a component of the TCC, an intermediate of the TCC, or to C5b-9. TCC components, TCC intermediates, and suitable epitopes to which the first or second antigen combining site can bind are described herein. In some embodiments, where the first antigen combining site binds to C5b, the component of the TCC to which the second antigen combining sites binds is not C5b. In some embodiments, the first antigen combining site binds to a first epitope of C5b and the second antigen combining site binds to a second epitope of C5b, wherein the first and second epitopes are not identical. For example, the first and second epitopes of C5b can be non-overlapping or can be composed of two different amino acid sequences.

In some embodiments, a bispecific antibody described herein can contain a first and second antigen combining site that each bind to the same target (e.g., C5a, C5b, C5aR1, C5L2, C5b-6, C5b-7, C5b-8, C5b-9, C6, C7, C8, or C9), wherein each antigen combining site binds to a different epitope of the target. For example, in some embodiments, a bispecific antibody described herein can contain a first antigen combining site that binds to C5a and a second antigen combining site that binds to
C5a at an epitope that is not identical to, or overlapping with, the epitope to which the first antigen combining site binds.

In some embodiments, a bispecific antibody described herein binds to the extracellular portion of the cellular receptor for C5a. For example, a bispecific antibody described herein can bind to the extracellular region of C5aR1 and/or C5L2.

In some embodiments, a bispecific antibody described herein can inhibit the interaction between C5a and a cellular receptor for C5a (e.g., C5aR1 or C5L2). In some embodiments, a bispecific antibody described herein can inhibit the assembly or activity of the TCC. In some embodiments, a bispecific antibody described herein can inhibit the C5a-dependent chemotaxis of a cell expressing a receptor for C5a. In some embodiments, a bispecific antibody described herein can inhibit the interaction between C5b and C6; C5b-6 and C7; C5b-7 and C8; or C5b-8 and C9. In some embodiments, a bispecific antibody described herein can inhibit complement-dependent lysis of a cell in vitro.

In some embodiments, a bispecific antibody described herein can bind to a cognate antigen with a $K_a$ of at least $10^8$ (e.g., at least $10^9$, $10^{10}$, or $10^{11}$) M$^{-1}$. For example, a bispecific antibody described herein can bind to C5a with a $K_a$ of at least $10^8$ M$^{-1}$. In another example, a bispecific antibody can bind to C5aR1 or C5L2 with a $K_a$ of at least $10^8$ M$^{-1}$. In yet another example, a bispecific antibody described herein can bind to C5b with a $K_a$ of at least $10^8$ M$^{-1}$. In yet another example, a bispecific antibody described herein can bind to C5b-6 with a $K_a$ of at least $10^8$ M$^{-1}$.

In some embodiments, a bispecific antibody described herein can further contain a third antigen combining site that binds to an antigen present in a terminal complement protein selected from the group consisting of C6, Cl, C8, C9. In some embodiments, a bispecific antibody can contain an antigen combining site that binds to full-length or mature C5.

In some embodiments, a bispecific antibody described herein can be monoclonal, single-chain, humanized, recombinant, chimeric, chimerized, deimmunized, fully human, a diabody, an intrabody, or an F(ab')$_2$ fragment. The bispecific antibody can be a single chain diabody, a tandem single chain Fv fragment, a tandem single chain diabody, or a fusion protein comprising a single chain diabody and at least a portion of an immunoglobulin heavy chain constant region. The bispecific antibody can be a dual variable domain immunoglobulin (DVD-Ig)
molecule. The bispecific antibody can contain, or be, two different monospecific antibodies that are associated with one another (e.g., covalently or non-covalently linked together).

In some embodiments, any bispecific antibody described herein can contain a heterologous moiety such as a sugar (e.g., an antibody can be glycosylated) or a detectable label. Detectable labels include, e.g., fluorescent labels, luminescent labels, heavy metal labels, radioactive labels, and enzymatic labels. A fluorescent label can be, e.g., selected from the group consisting of fluorescein, fluorescein isothiocyanate (FITC), green fluorescent protein (GFP), DyLight 488, phycoerythrin (PE), propidium iodide (PI), PerCP, PE-Alexa Fluor® 700, Cy5, allophycocyanin, and Cy7. An enzymatic label can be, e.g., horseradish peroxidase, alkaline phosphatase, or luciferase. A radioactive label can be, e.g., one selected from the group consisting of $^{32}\text{P}$, $^{33}\text{P}$, $^{14}\text{C}$, $^{125}\text{I}$, $^{131}\text{I}$, $^{35}\text{S}$, and $^{3}\text{H}$.

In another aspect, the disclosure features a pharmaceutical composition comprising any of the bispecific antibodies described herein and a pharmaceutically-acceptable carrier, excipient, or diluent.

In yet another aspect, the disclosure features a method for inhibiting or preventing terminal complement in a subject. The method includes the step of administering to a subject in need thereof an antibody in an amount effective to inhibit terminal complement in the subject, wherein the antibody is any bispecific antibody described herein capable of inhibiting terminal complement. For example, the bispecific antibody can be one that binds to a component of the TCC (e.g., C5b, C6, C7, C8, or C9), an intermediate of the TCC (e.g., C5b-6, C5b-7, or C5b-8), or to C5b-9.

In another aspect, the disclosure features a method for inhibiting or preventing C5a-dependent chemotaxis in a subject. The method includes the step of administering to a subject in need thereof an antibody in an amount effective to inhibit C5a-dependent chemotaxis in a subject. The antibody can be any bispecific antibody described herein capable of inhibiting C5a-dependent chemotaxis. For example, the bispecific antibody can be one that binds to C5a or C5aR1. The antibody can be administered as a pharmaceutical composition.

In another aspect, the disclosure provides a method for treating or preventing a complement-associated disorder in a subject, which method includes administering to a
subject in need thereof an antibody in an amount effective to treat a complement-associated disorder in the subject. The antibody can be, e.g., a bispecific antibody described herein. For example, the antibody can be one that binds to: (i) C5a and C5b; (ii) C5b and a receptor for C5a; (iii) C5a and a component or intermediate of the TCC (or C5b-9); (iv) a receptor for C5a and a component or intermediate of the TCC (or C5b-9); or (v) C5b and a component or intermediate of the TCC (or C5b-9). The antibody can be administered as a pharmaceutical composition.

In some embodiments of any of the methods described herein, the subject can one having, suspected of having, or at risk for developing a complement-associated disorder. The complement-associated disorder can be, e.g., an alternative complement pathway-associated disorder such as, e.g., rheumatoid arthritis, asthma, ischemia-reperfusion injury, atypical hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, paroxysmal nocturnal hemoglobinuria, dense deposit disease, age-related macular degeneration, spontaneous fetal loss, Pauci-immune vasculitis, epidermolysis bullosa, recurrent fetal loss, multiple sclerosis, traumatic brain injury, or any other AP-associated disorder described herein or known in the art.

In some embodiments, the subject has, is suspected of having, or at risk for developing a classical complement pathway-associated disorder such as, e.g., Hashimoto's thyroiditis, type I diabetes, psoriasis, pemphigus, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, Goodpasture syndrome, antiphospholipid syndrome, catastrophic antiphospholipid syndrome, or any other CP-associated disorder described herein or known in the art of medicine.

Any of the methods described herein can further include the step of identifying the subject as having, suspected of having, or at risk for developing a complement-associated disorder. Any of the methods can include, after administering the antibody, monitoring the subject for an improvement in one or more symptoms of the complement-associated disorder.

In some embodiments of a method described herein, the antibody can be intravenously administered to the subject. In some embodiments (e.g., in embodiments where a respiratory condition is to be treated), the antibody can be administered to the subject by way of the lungs. In some embodiments, the antibody can be administered subcutaneously or intramuscularly.
In some embodiments of a method described herein, the subject is a mammal.
In some embodiments, the subject is a human.

In yet another aspect, the disclosure also features a method for producing any of the bispecific antibodies described herein. The method can include, e.g., culturing a cell that expresses a bispecific antibody under conditions that allow for the antibody to be expressed by the cell. The method can also include isolating the bispecific antibody from the cell or from the media in which the cell is cultured. The cultured cell can contain a nucleic acid expression vector containing a nucleotide sequence encoding the bispecific antibody. The vector can be integrated into the cell genome or can be episomal. The cell can be, e.g., a bacterial cell, a fungal cell (e.g., a yeast cell), an insect cell, or a mammalian cell. The cell can be a human cell. In some embodiments, methods for producing a bispecific antibody described herein involve chemically synthesizing the antibody.

In another aspect, the disclosure features a population of cultured cells, a plurality of which express a bispecific antibody described herein. The population can include two or more pluralities of cells, each plurality including cells that express a different bispecific antibody.

As used herein, "associated with" in the context of an interaction between two or more atoms or molecular units (e.g., between two different monospecific antibodies or antigen-binding fragments of the monospecific antibodies), includes any covalent or non-covalent bonding, or physical admixture, of two or more atoms or molecular units. The chemical nature of covalent bonds (two atoms sharing one or more pairs of valence electrons) are known in the art and include, e.g., disulfide bonds or peptide bonds. A non-covalent bond is a chemical bond between atoms or molecules that does not involve the sharing of pairs of valence electrons. For example, non-covalent interactions include, e.g., hydrophobic interactions, hydrogen-bonding interactions, ionic bonding, Van der Waals bonding, or dipole-dipole interactions. Examples of such non-covalent interactions include, e.g., binding pair interactions (interactions of a first and second member of a binding pair such as the interaction between streptavidin and biotin). For example, a bispecific antibody can contain, or be, a first monospecific antibody and a second monospecific antibody non-covalently linked together by way of an avidin/streptavidin binding pair.
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 complement-associated disorders in a subject, will be apparent from the following description, the examples, and from the claims.

Brief Description of the Drawings

Fig. 1 is a line graph depicting the time-dependent beta-phase clearance of a humanized anti-human C5 antibody in a human Fc receptor of neonate (FcRn) mouse model. The Y-axis represents the percentage of the initially administered amount of the humanized antibody that remains in the serum of the mice. The X-axis represents the time in days.

Fig. 2 is a schematic diagram depicting the basic pathways for clearance of a humanized anti-human C5 antibody and its target antigen C5 in patients. Free antibody "A" refers to free (uncomplexed) antibody and "C" refers to uncomplexed or free C5. The antibody:C5 complex is represented by "CA." The rate constant for the association of the antibody and C5 is represented by "£3" and the rate constant for the dissociation of the complex is represented by "k4." The antibody:C5 complex can be eliminated by immune complex clearance having a rate constant represented by "k6." Free antibody is also eliminated as represented by a different rate constant "k5." C5 is constitutively expressed with a rate constant kl and it is eliminated with a rate constant of "k2."

Fig. 3 is a schematic diagram depicting a simplified series of pathways for clearance of a humanized anti-human C5 antibody in patients. "mAb" refers to the humanized antibody. "C" refers to free C5. In this simplified pathway, the immune complex clearance has a rate constant "kl." Free antibody clearance is governed by the first order equation \[ A = A_0 \times e^{-kt} \] (Equation 2; see below), where "t" represents
time and "£8" is the rate constant. Immune complex clearance is governed by the zero order equation \( A = A_0 - kit \) (Equation 3; see below), where \( A_0 \) is the concentration of the free antibody at time 0. The integrated rate equation for the concurrent processes is set forth as \( A = A_0 \times e^{(-kit)} \) (Equation 4; see below).

**Detailed Description**

The disclosure provides bispecific antibodies that are useful for inhibiting one or both of terminal complement (e.g., the assembly and/or activity of the C5b-9 TCC) and C5a anaphylatoxin-mediated inflammation. While in no way intended to be limiting, exemplary bispecific antibodies, conjugates, pharmaceutical compositions and formulations, and methods for using any of the foregoing are elaborated on below and are exemplified in the working Examples.

**Antibodies**

A bispecific antibody molecule described herein is one that binds to two or more (e.g., two, three, four, five, or six or more) different epitopes. For example, a bispecific antibody can bind to two or more (e.g., two, three, four, five, or six or more) different proteins, wherein at least two of the proteins are selected from C5a, C5b, a receptor for C5a (e.g., C5aRI or C5L2), and a component or intermediate of the TCC such as C5b-6, C5b-7, C5b-8, or C5b-9. For example, the bispecific antibody can be one that binds to C5a and C5b. The bispecific antibody can be one that binds to C5aR and C5a. In another example, the bispecific antibody can be one that binds to C5aRI and C5b. In some embodiments, a bispecific antibody described herein binds to two different proteins selected from the group consisting of C5a, C5aRI, C5L2, C5b, C5b-6, C5b-7, C5b-8, and C5b-9. In some embodiments, the bispecific antibody does not bind to full-length or mature C5.

The antibodies described herein can be used in a number of diagnostic and/or therapeutic applications. For example, a bispecific antibody described herein that binds to C5b, C5b-6, C5b-7, C5b-8, and/or C5b-9 is useful for inhibiting terminal complement *in vitro* or *in vivo*. In some embodiments, an antibody that binds to one or both of C5a and C5aRI is useful for inhibiting a C5a anaphylatoxin-associated inflammatory response. Accordingly, the antibodies described herein are useful for preventing, treating, or ameliorating one or more symptoms of a variety of
complement-associated conditions in a subject such as, but in no way limited to:
PNH, hemolytic uremia syndrome (HUS), AMD, asthma, and sepsis.

In some embodiments, at least one antigen combining site of a bispecific antibody described herein binds to C5a, but not to full-length, native C5. Full-length C5 (e.g., the amino acid sequence of C5) is described in, e.g., Haviland et al. (1991) J Immunol 146(1):362-8. (See also Genbank Accession No. AAA5 1925.) Pro-C5 is a 1676 amino acid residue precursor protein. 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, the antigen combining site that binds to C5a also binds to desarginated C5a. In some embodiments, the antigen combining site that binds to C5a does not bind to desarginated C5a.

In some embodiments, at least one antigen combining site of a bispecific antibody described herein can bind 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)
In another example, a bispecific 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), 152-1486 (BD Pharmingen/BD Biosciences), abl 1877 (Abeam, Cambridge, Massachusetts), and HM2079 (clone 2952; HyCult Biotechnology, the Netherlands). In some embodiments, the bispecific antibody that binds to C5a can crossblock the binding of any of the aforementioned C5a neoepitope-specific antibodies.

As used herein, the term "crossblocking antibody" refers to a subject bispecific antibody that lowers the amount of binding of a reference antibody to an epitope relative to the amount of binding of the reference antibody to the epitope in the absence of the subject antibody. Suitable methods for determining whether a subject bispecific antibody crossblocks the binding of a reference antibody to an epitope are known in the art.

In some embodiments, at least one antigen combining site of a bispecific antibody described herein can bind to a mammalian (e.g., human) form of C5a. For example, the antigen combining site can bind to a human C5a protein having the following amino acid sequence:

TLQKKIEIAAKYKHVVVKCCYDGACVNNDETCEQRAARISLGPRCIKAFTECCVVASQLRANISHKDMQLGR (SEQ ID NO: 1). The antigen combining site can bind to human C5a at an epitope within or overlapping with the amino acid sequence: CCYDGACVNNDETCEQRAAR (SEQ ID NO:2); KCCYDGACVNNDETCEQR (SEQ ID NO:3); VNNDETCEQR (SEQ ID NO:4); VNNDET (SEQ ID NO:5); AARISLGPR (SEQ ID NO:6); CCYDGACVNNDETCEQRAA (SEQ ID NO:7); CCYDGACVNNDETCEQRA (SEQ ID NO:8); CCYDGACVNNDETCEQR (SEQ ID NO:9); CCYDGACVNNDETCEQ (SEQ ID NO:10); CCYDGACVNNDETCE (SEQ ID NO:11); CYDGACVNNDETCEQRAAR (SEQ ID NO: 12); YDGACVNNDETCEQRAAR (SEQ ID NO:13); or CYDGACVNNDETCEQRAAR (SEQ ID NO:14). In some embodiments, the antigen combining site can bind to 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: 1-14. 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.

The "antigen combining site" of an antibody, as used herein, refers to a surface of an antibody molecule that makes specific, physical contact with an antigen. The antigen combining site is typically composed of six hypervariable loops or complementarity determining regions (CDRs) within the hypervariable region of an immunoglobulin, three CDRs from the light chain and three CDRs from the heavy chain of the immunoglobulin molecule. The exact boundaries of CDRs within the hypervariable regions of light and heavy chain polypeptides have been defined differently according to different methods. In some embodiments, the positions of the CDRs within a light or heavy chain variable domain can be as defined by Kabat et al. (1991) "Sequences of Proteins of Immunological Interest." NIH Publication No. 91-3242, U.S. Department of Health and Human Services, Bethesda, MD. In such cases, the CDRs can be referred to as "Kabat CDRs" (e.g., "Kabat LCDR2" or "Kabat HCDR1"). In some embodiments, the positions of the CDRs of a light or heavy chain variable region can be as defined by Chothia et al. (1989) Nature 342:877-883. Accordingly, these regions can be referred to as "Chothia CDRs" (e.g., "Chothia LCDR2" or "Chothia HCDR3"), respectively.

In some embodiments, the binding of a bispecific 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(6):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 C5aRI. Suitable methods for detecting and/or measuring the interaction between C5a and C5aRI (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(2): 149-154; Giannini et al. (1995) J Biol Chem 270(32): 19166-19172; and U.S. Patent No. 20060160726. For example, the binding of detectably labeled (e.g., radioactively labeled) C5a to C5aRI-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 C5aRl 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 C5aRl. 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, at least one antigen combining site of a bispecific antibody described herein binds to C5b, but does not bind to full-length, native 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*. 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 includes C5b-6 (including C5b and C6), C5b-7 (including C5b, C6, and Cl), 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) that mediate rapid osmotic lysis of the target cells.

In some embodiments, the binding of a bispecific antibody described herein 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 the bispecific 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 Abeam™ (ab46151 or ab46168).

In some embodiments, at least one antigen combining site of a bispecific antibody described herein can bind to a mammalian (e.g., human) form of C5b. For example, the antigen combining site can bind to a portion of a human C5b protein having the following amino acid sequence:

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QEQTYVISAPKIFRVGASENIVIQVYGYTEAFDATISIKSYDPDKKFSYSSGHVHL
SSENKFQNSAILTIQPKQLPGGPVQYVYLEVSHKFSKSKRMPITYDNGFLFIHTDKPVYTPQSVKVRVYSLNDDLKLPAKRETRTLTFIDPEGSEVDVMVEEIDHI
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GIISFPDFKIPSNPRYGMWTIAKYKEDFSTTTGTAYFEVKVEYLPHEFSVSIPEY
NFIGYKNFKNFIEITAKRYFYNKVTEADVYITFGIREDLKDDQKEMMQTAM

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QNTMLINGIAQVTFDSETAVKELSYYSLEDLNKYLITYAVTIESTGGFSEEAIEPGIKYVLSPYKLNLVATPLFLKPGIPYPKVKVQVKSVDLQLVGGVLPVMNAQTID
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VNQETSDLPSKVSSTVDDGVASFVLNLPSGVTVLEFVVTDDAPDLEENQA
REGYRAIAAYSSLQSYYYIDWTDNHKALLVGEHLNIIVTPKSPYIDKITHYNYL
ILSKGKIIIFGTREKFSDASYQSINIPVTQNMVSPRSSLLVVYIVTGEQTAELVSD

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SVWLNIEEKCNGQLQVHLSPDADAYSQGQTSLNMATGMDSWVALAADVSA
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VYGVQVRGAKKPLERVFQFLEKSDLGCAGGGLNNANVFHLAGLTFLTNNADDSEQENDPCEKIEL (SEQ ID NO: 15). The antigen combining site can bind to a portion of a human C5b protein having the following amino acid sequence:

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LHMKTLIPVSKPEISYFEPWLSWEHVLVPRRQLQFAIPDSLWTTEIQGIGIS
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NTGICVADTVKAVFVDFLEMNIPSYVVRGEQIQLKTGTVYNYRTSGMQFCV
KMSAVEGICETSESPVIDHQGTKSSKCRQKVEGSSSHLVTFTVLIEGLHVIN
ESLETWFGKEILVKTTRVPEGKRESYSVTSPLPRGITYGTISRRKEFYIRPL
DLVPKTEIKRISVKGFLVEILAIVSLSQEGINILTHPKGSAEAELMSVVPVVFY
VFHYLETGNHNIFSHDPIEIKQLKKKLKEGMLSIMSRNADYSVWVKI

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GASTWLTAFALRVLQGVQNVKSYEQNSICNSLLWLVENYQILDSFKENS
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QUQPIKLGTLVEARENLYLTAFTVGIRKAFCIPPLVKIDTALIKADNFLLE
NTLPAQSTFTLAIASAYALSMDKTQMPQRSIVSALKREALVKGNPPIYRFWKD
NLQHKDSSVPNTGTMVETTAYALLTSLNLKDINYVNPIKWLSEEQRYGG
GFYSTQDTAINAEGLTEYSLLVKQLRLSMDIDVSYKHKGALHNYKMTDKNFL  
GRPVEVLLNDDLDLVSTGFSGLATVHVTVTVKYSTSEEVCSFYLKDIDQDIEASHYRGYNSDYKRIVACASYKPSREESSSSGSSHAVMDISLPTGISANEEDLKA  
LVEGVDQLFTDYQIKDGHVILQLNSIPSS  
DFLCVRFIFELEFVEGFLSPATFTVYEYHRPDQCTMFYSTSNIKIQKVCEGAA  
CKCVEADCGQMEEELDLTISAEARKQTACKPEIAYAYKVSITSITVENFVKY  
KATLLDIYKTGEAVAEKDSEITFIKKTCTNAELVKGRQYLIMGKEALQIKYNFSFRYIYPLDSLTWIEYWPRTTCCSSCQAFLANLDEFAEDIFLNGC (SEQ ID NO: 16). In some embodiments, the antigen combining site 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:15 or SEQ ID NO:16.

Additional exemplary sub-fragments of human C5b or C5a to which a bispecific antibody described herein 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, at least one antigen combining site of a bispecific antibody described herein binds to a receptor for human C5a. For example, at least one antigen combining site of the antibody can bind to C5aR (C5aRI or CD88) or to C5L2 (GPR77). In some embodiments, at least one antigen combining site of a bispecific antibody described herein binds to C5aRI (e.g., to the extracellular region of C5aRI). C5aRI has been described in detail in, e.g., Gerard and Gerard (1991) Nature 349(6310):614-617; Bao et al. (1992) Genomics 13:437-440; and U.S. Patent Application Publication No. 20050244406. For example, C5aRI belongs to the family of seven transmembrane G-protein-coupled receptors and binds with high affinity to C5a. The C5a/C5aRI interaction has a $K_d$ of about 1 nM. C5aRI contains an extended N-terminal extracellular domain; the C5aRI structure conforms to the seven transmembrane receptor family, with the extracellular N-terminus being followed by seven transmembrane helices connected by interhelical domains alternating as intracellular and extracellular loops, and ending with an intracellular C-terminal domain. C5aRI can be found on many cell types including, e.g., smooth muscle cells, endothelial cells, and a variety of lymphocytes including, without
limitation, neutrophils and macrophages. (See, e.g., Soruri et al. (2003) *Immunol Lett* 88(1):47-52; Zwirner et al. (1999) *Mol Immunol* 36(13-14):877-884; and Kiafard et al. (2007) *Immunobiology* 212(2):129-139. The binding of C5a to C5aR (C5aR1 or CD88) triggers a number of pro-inflammatory effects including, e.g., chemotaxis of several myeloid lineage cells (e.g., neutrophils, eosinophils, basophils, macrophages, and monocytes) and increased vascular permeability. High level activation of C5aR1 results in lymphocyte degranulation and activation of NADPH oxidase. C5a/C5aR1 have been implicated in the pathogenesis of a number of disorders including, e.g., sepsis, septic shock, SIRS (systemic/severe inflammatory response syndrome), MOF (multi organ failure), ARDS (acute respiratory distress syndrome), rheumatoid arthritis, and recurrent pregnancy loss. (See, e.g., Rittirsch et al. (2008) *Nature Med* 14:551-557; Girardi et al. (2003) *J Clin Invest* 112:1644-1654; Atkinson (2003) *J Clin Invest* 112:1639-1641; U.S. Patent No. 7,455,837; U.S. Patent Application Publication Nos. 20070065433, 20070274989, and 20070123466, the disclosures of each of which are incorporated herein by reference in their entirety.) An exemplary amino acid sequence for human C5aR1 is provided herein and set forth in, e.g., Genbank Accession No. NP_001727.

In some embodiments, at least one antigen combining site of a bispecific antibody described herein can bind to a mammalian (e.g., human) C5aR1. The human C5aR1 can have, e.g., the following amino acid sequence:

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MNSFNYTTTPDYGHYDDKTDLLNTVPDKTSNTLVRPDLALVIFAVVFLVGV
LGNALVVWVTAFEAKRINTIAIWFLNLAVADFLSCLAPLFLTSIVQHHHWPG
GAACSILPSULLMYASILLALLATISADFLLL VFKPIWCQNFRGAGLAWIACAV
AWGLALLTIPSFLYRVVREEFYPKKVLCLGVDYSHDKRRERAVAIWRLVGLFL
WPLLTLTICYTFILLRTWSRRATRSTKTLKVVAVVASFFIFWLPYQVTGIMM
SFLEPSSPTFLLLNKDLSCSFAYINCCINPIYVVAQGFQGLRKLKSLPSLLR
NLTEESVRESKSFSTRSTVTMAQKTQAV (SEQ ID NO: 17). In some embodiments, the antigen combining site can bind to human C5aR1 at an epitope that is within or overlapping with the amino acid sequence: SIVQQHHHWPGAAAC

(SEQ ID NO: 18); RVVREEFYPKKVLCLGVDYSHDKRRERAVAIWRLVGLFL
WPLLTLTICYTFILLRTWSRRATRSTKTLKVVAVVASFFIFWLPYQVTGIMM
SFLEPSSPTFLLLNKDLSCSFAYINCCINPIYVVAQGFQGLRKLKSLPSLLR
NLTEESVRESKSFSTRSTVTMAQKTQAV (SEQ ID NO: 17). In some embodiments, the antigen combining site can bind to human C5aR1 protein or fragment thereof containing, or consisting 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: 17-20.

In some embodiments, at least one antigen combining site of a bispecific antibody described herein binds to the amino terminus of human C5aR. For example, the antigen combining site can bind to an epitope that is within or overlapping with the amino acid sequence:

MNSFYTTDPYGHDDKDTLDLNTPVDKT (SEQ ID NO:21) or
DYGHYDDKDTLDLNTPVDKT (SEQ ID NO:22). In some embodiments, the antigen combining site binds to a human C5aR protein containing, or consisting 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 NO: 21 or 22. In some embodiments, an antigen combining site binds to a human C5aR protein at an epitope within or overlapping with an amino acid sequence containing amino acids 24-30 of SEQ ID NO:17.

The binding of an antibody to C5aR can, in some embodiments, antagonize the activity of C5aR. In some embodiments, the binding of the bispecific antibody to C5aR can inhibit the interaction between C5a and C5aR. Methods for detecting and/or measuring the interaction between C5a and C5aR are described herein. Methods for measuring the activity of C5aR (or inhibition thereof) are known in the art and include, e.g., a C5a-directed *in vitro* neutrophil chemotaxis assay as described in, e.g., U.S. Patent Application Publication No. 20050244406.

Exemplary C5aR antibodies, as well as methods for making the antibodies, are described in, e.g., U.S. Patent Application Publication No. 20050244406 and U.S. Patent No. 7,455,837, the disclosure of each of which is incorporated herein by reference in its entirety.

In some embodiments, at least one antigen combining site of a bispecific antibody described herein binds to the C5a receptor C5L2. C5L2 is a high affinity receptor for C5a and is expressed on, e.g., granulocytes and immature dendritic cells. Unlike C5aR, C5L2 is not coupled to G proteins. (See, e.g., Monk et al. (2007) *Br J Pharm* 152:429-448 and Huber-Lang et al. (2005) *J Immunol* 174:1 104-1 110.) In some embodiments, at least one antigen combining site of a bispecific antibody described herein can bind to a mammalian (e.g., human) C5L2. The human C5L2 can have, e.g., the following amino acid sequence:
MGNDSVSYEYGDYSLSRDPVDCLDGACLAIDPLRVAPLPLYAAIFLVGVPG
NAMVAWVAGKVARRVGATWLHLAVADLLCCLSLPILAVPIARGGHWPYGAVGCRALPSIILLTM ...
NAMVAWVAGKVARRRVGATWLLHLAVADLLCCLSLPILAVPIARGGHWPY
GAVGCRALPSIILLTM ... e.g., C5b-6, C5b-7, C5b-8, and
C5b-9. Thus, in some embodiments, the antigen combining site can bind to the C5b-6
presented
presented
measuring
bind
AAPNSALLARALRAEPLIVGLALAHSCLNPMLFLYFGRAQLRRSLP
binding
inhibit
MGNDSVSYEYGDYSDLSDRPVDCLDGACLAIDPLRVAPLPLYAAIFLVGVPG
LTVAAPNSALLARALRAE (SEQ ID NO:26) or
MGNDSVSYEYGDYSDLSDRPVDCLDGACLAIDPL (SEQ ID NO:27). In some
embodiments, the antigen combining site can bind to human C5L2 protein or
fragment thereof containing, or consisting 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 SEQ ID NO:23. In some embodiments, the antigen combining site can
bind to human C5L2 at an epitope that is within or overlapping with the amino acid
sequence: PIARGGHWPYGAVGCR (SEQ ID NO:24);
RRLHQEHFPARLQCVVDYGSSSTENAVTAIR (SEQ ID NO:25);
LTVAAPNSALLARALRAE (SEQ ID NO:26) or
MGNDSVSYEYGDYS (SEQ ID NO:27). In some
embodiments, the antigen combining site can bind to human C5L2 protein or
fragment thereof containing, or consisting 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:24-27.

In some embodiments, the binding of the bispecific antibody to C5L2 can
inhibit the interaction between C5a and C5L2. Methods for detecting and/or
measuring the interaction between C5a and C5L2 are described herein and in, e.g.,
Chem 277(9):7165-7169.

In some embodiments, at least one antigen combining site of a bispecific
antibody described herein binds to a component of the terminal C5b-9 complement
complex (TCC), e.g., C5b, C6, C7, C8, or C9. In some embodiments, at least one
antigen combining site of a bispecific antibody described herein binds to a neoepitope
present in an intermediate of the TCC or of the TCC itself, i.e., a neoepitope that is
presented to solvent upon formation of an intermediate of the TCC or the TCC itself.
As described above, intermediates of the TCC include, e.g., C5b-6, C5b-7, C5b-8, and
C5b-9. Thus, in some embodiments, the antigen combining site can bind to the C5b-6
intermediate, but not to uncomplexed C5b or to uncomplexed C6. In some
embodiments, the antigen combining site binds to the C5b-6 intermediate, but does
not bind to C5b-7, C5b-8, C5b-9, or any combination of the foregoing. In some
embodiments, the binding of the antibody to C5b-6 inhibits the interaction between
C5b-6 and C7. In some embodiments, the binding of the antibody to C5b-6 inhibits
the formation of the TCC. Suitable methods for detecting the interaction between
various members of the TCC and/or its intermediates (e.g., C5b-6 and C7) are
or activity of the TCC are well known in the art and include, e.g., hemolytic assays
and use of C5b-9 neoepitope-specific antibodies.

Antibodies that bind to neoepitopes present on the C5b-6 intermediate, as well
as methods for generating and identifying such antibodies, have been described in,
e.g., Podack et al. (1978), supra and Mollnes et al. (1989) Complement Inflamm
6(3):223-235.

In some embodiments, an antigen combining site of the bispecific antibody
binds to a neoepitope present in C5b-7, a complex containing C5b, C6, and C7. In
some embodiments, an antigen combining site of the bispecific antibody binds to a
neoepitope present in C5b-8, a complex containing C5b, C6, C7, and C8. The
foregoing antibodies, as well as methods for producing the antibodies, are described
in, e.g., Mollnes et al. (1989), supra. The antibodies can be useful to inhibit the
assembly and/or activity of the TCC.

In some embodiments, any of the bispecific antibodies described herein can
contain a third antigen combining site that binds to a terminal complement protein
selected from the group consisting of C6, C7, C8, C9, and full-length, native C5. In
some embodiments, any of the bispecific antibodies described herein can contain at
least one antigen combining site that binds to: (i) C5a and C5 or (ii) C5b and C5. In
other words, an antigen combining site that binds to C5a can also bind to full length
or mature C5 and an antigen combining site that binds to C5b can also bind to full
length or mature C5.

In some embodiments, a bispecific antibody specifically binds to C5a, C5b,
and/or C5aR. The term "specific binding" or "specifically binds" refers to two
molecules forming a complex (e.g., a complex between a bispecific antibody and C5a, C5b, or C5aR) 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, a bispecific antibody can specifically bind to a protein (e.g., C5a, C5b, or C5aR) 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.


As used herein, the term "bispecific antibody" refers to a whole or intact antibody molecule (e.g., IgM, IgG (including IgG1, IgG2, IgG3, and IgG4), IgA, IgD, or IgE) and any fragment thereof, which binds to two or more different proteins, at least two of which being C5a, C5b, or C5aR (see above). The term bispecific antibody includes, e.g., a chimerized or chimeric antibody, a humanized antibody, a deimmunized human antibody, and a fully human antibody. Bispecific antibodies also include, e.g., F(ab')_2 fragments or conjugates of two or more monospecific antibody fragments (e.g., two or more scFv, a Fab, an Fab', or an Fd immunoglobulin fragment). In addition, bispecific intrabodies, minibodies, triabodies, and diabodies (see, e.g., Todorovska et al. (2001) J Immunol Methods 248(1) :47-66; Hudson and Kortt (1999) J Immunol Methods 231(1):177-189; Poljak (1994) Structure 2(12):1121-1123; Rondon and Marasco (1997) Annual Review of Microbiology
5J::257-283, the disclosures of each of which are incorporated herein by reference in their entirety) are also included in the definition of bispecific antibody and are compatible for use in the methods described herein. Also embraced by the term bispecific antibody are tandem single chain antibodies, single chain diabodies, tandem single chain diabodies, and fusion proteins containing single chain diabodies and at least a portion of a heavy chain constant region (e.g., a CH1 or a CH3 region of a heavy chain polypeptide) as described in, e.g., Kontermann (2005) Acta Pharmacologica Sinica 26(1): 1-9; Kufer et al. (2004) Trends Biotechnol 22:238-244; and Kriangkum et al. (2001) Biomol Eng ±8:31-40.

Also embraced by the term “bispecific antibody” are antibodies containing at least one antigen combining site comprised of fewer than six canonical CDRs, e.g., a bispecific antibody where the binding specificity of one antigen combining site is determined by three, four or five CDRs, rather than six. Examples of antibodies wherein binding affinity and specificity are contributed primarily by one or the other variable domain are known in the art. Jeffrey et al. [(1993) Proc Natl Acad Sci USA 90: 10310-10314] discloses an anti-digoxin antibody that binds to digoxin primarily by the antibody heavy chain. Accordingly, a skilled artisan can identify an antibody containing a single variable domain and that binds to, e.g., C5a, C5b, or C5aR in accordance with the disclosure.


A wide variety of bispecific antibody formats are known in the art of antibody engineering and methods for making the bispecific antibodies are well 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.
the desired binding specificities (antibody-antigen combining sites) can be fused to
immunoglobulin constant domain sequences. The fusion can include an
immunoglobulin heavy-chain constant domain, e.g., at least part of the hinge, CH2,
and CH3 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.
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.

U.S. Patent No. 5,534,254 describes several different types of bispecific
antibodies including, e.g., single chain Fv fragments linked together by peptide
couplers, chelating agents, or chemical or disulfide couplings. In another example,
methods for chemically cross-linking two monospecific antibodies to thus form a
bispecific antibody. As described above, a bispecific antibody described herein can
be formed, e.g., by conjugating two single chain antibodies which are selected from,
e.g.: (a) an antibody specific for a C5a; (b) an antibody specific for C5b; (c) an
antibody specific for C5aRI; (d) an antibody specific for C5L2; (e) an antibody
specific for C5b-6; (f) an antibody specific for C5b-7; (g) an antibody specific for
C5b-8; and (h) an antibody specific for C5b-9. A bispecific antibody, in some
embodiments, can be a fusion protein containing a monoclonal antibody to C5a or
C5b (or an antigen-binding fragment thereof) and an antibody or antigen-binding
fragment thereof specific to C5aRI. In some embodiments, the bispecific antibody is
a fusion protein containing a monoclonal antibody or fragment thereof specific to C5a
and a second monoclonal antibody or fragment thereof that is specific to C5b. In
some embodiments, the bispecific antibody is a fusion protein containing a monoclonal antibody or fragment thereof specific for a component or intermediate of the TCC and a monoclonal antibody that is specific for C5a or a receptor for C5a (e.g., C5aR1 or C5L2).

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 and de Kruijff and Logtenberg (1996) *J Biol Chem* 271(13):7630-7634.) 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.

In some embodiments, the bispecific antibody can be a tandem single chain (sc) Fv fragment, which contain two different scFv fragments covalently tethered together by a linker (e.g., a polypeptide linker). See, e.g., Ren-Heidenreich et al. (2004) *Cancer* 100:1095-1103 and Korn et al. (2004) *J Gene Med* 6:642-651. In some embodiments, the linker can contain, or be, all or part of a heavy chain polypeptide constant region such as a CH1 domain as described in, e.g., Grosse-Hovest et al. (2004) *Proc Natl Acad Sci USA* 101:6858-6863. In some embodiments, the two antibody fragments can be covalently tethered together by way of a polyglycine-serine or polyserine-glycine linker as described in, e.g., U.S. patent nos. 7,12,324 and 5,525,491, respectively. See also U.S. patent no. 5,258,498, the disclosure with respect to antibody engineering and linkers is incorporated herein by reference in its entirety. Methods for generating bispecific tandem scFv antibodies are described in, e.g., Maletz et al. (2001) *Int J Cancer* 93:409-416; Hayden et al. (1994) *Ther Immunol* 1:3-15; and Honemann et al. (2004) *Leukemia* 18:636-644. Alternatively, the antibodies can be "linear antibodies" as described in, e.g., Zapata et al. (1995) *Protein Eng.* 8(00): 1057-1062. Briefly, these antibodies comprise a pair of tandem Fd segments (V_h-C_H1-V_h-C_H1) that form a pair of antigen binding regions.

A bispecific antibody can also be a diabody. Diabody technology described by, e.g., 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. (See also, e.g., Zhu et al. (1996) Biotechnology
chain diabodies (scDb) as well as methods for generating scDb are described in, e.g.,

The disclosure also embraces variant forms of bispecific antibodies such as the
tetraivalent 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. For example, the
DVD-Ig light chain polypeptide can contain in tandem: (a) a VL from an antibody
that binds to C5a; and (b) a VL from an antibody that binds to C5b. Similarly, the
heavy chain comprises two different heavy chain variable domains (VH) linked in
tandem, followed by the constant domain CH1 and Fc region. For example, the
DVD-Ig heavy chain polypeptide can contain in tandem: (a) a VH from an antibody
that binds to C5a; and (b) a VH from an antibody that binds to C5b. In this case,
expression of the two chains in a cell results in a heterotetramer containing four
antigen combining sites, two that specifically bind to C5a and two that specifically
bind to C5b. It is understood that VL or VH from antibodies that bind to a receptor
for C5a (e.g., C5aR1 or C5L2) or a component or intermediate of the TCC (e.g., C5b-
6, C5b-7, C5b-8, or C5b-9) can also be used in the preparation of a DVD-Ig molecule
in accordance with the disclosure. 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. Also embraced is the bispecific format described
in, e.g., U.S. patent application publication no. 20070004909, the disclosure of which
is incorporated by reference in its entirety.

The antibodies (e.g., monoclonal antibodies) or fragments thereof that are used
to form the bispecific antibody molecules described herein can be, e.g., chimeric
antibodies, humanized antibodies, rehumanized antibodies, deimmunized antibodies, fully human antibodies, and antigen-binding fragments thereof. Chimeric antibodies are produced by recombinant processes well known in the art of antibody engineering and have a non-human mammal variable region and a human constant region. However, the term "human

Humanized antibodies correspond more closely to the sequence of human antibodies than do chimeric antibodies. Humanized variable domains are constructed in which amino acid sequences of one or more CDRs of non-human origin are grafted to human framework regions (FRs) as described in, e.g., Jones et al. (1996) Nature 321:522-525; Riechman et al. (1988) Nature 332:323-327 and U.S. Patent No. 5,530,101.

The humanized antibody can be an antibody that contains one or more human framework regions that are not germline. For example, the humanized antibody can contain one or more framework regions that were subject to somatic hypermutation and thus no longer germline per se. (See, e.g., Abbas, Lichtman, and Pober (2000) "Cellular and Molecular Immunology," 4th Edition, W.B. Saunders Company (ISBN:0721682332)). In some embodiments, the humanized antibody contains human germline framework regions, e.g., human germline V_H regions, human germline D regions, and human germline J regions (e.g., human germline J_H regions). The MRC Center for Protein Engineering maintains the online VBase database system, which includes amino acid sequences for a large number of human germline framework regions. See, e.g., Welschof et al. (1995) J Immunol Methods 179:203-214; Chothia et al. (1992) J Mol Biol 227:776-798; Williams et al. (1996) J Mol Biol 264:220-232; Marks et al. (1991) Eur J Immunol 21:985-991; and Tomlinson et al. (1995) EMBOJ. 14:4628-4638. Amino acid sequences for a repertoire of suitable human germline framework regions can also be obtained from the JOINSOLVER® Germline Databases (e.g., the JOINSOLVER® Kabat databases or the JOINSOLVER® IMGT databases) maintained in part by the U.S. Department of Health and Human Services and the National Institutes of Health. See, e.g., Souto-Carneiro et al. (2004) J Immunol. 172:6790-6802.

Fully human antibodies are 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 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 unarranged 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., C5a, C5b, or C5aR).

The wholly and partially human antibodies described above are less immunogenic than their entirely murine or non-human-derived antibody counterparts. All these molecules (or derivatives thereof) are therefore less likely to evoke an immune or allergic response. Consequently, they are better suited for in vivo administration in humans, especially when repeated or long-term administration is necessary, as may be needed for treatment with the bispecific antibodies described herein.

Methods for Producing a Bispecific Antibody

As described above, the bispecific antibodies can be produced using a variety of techniques known in the art of molecular biology and protein chemistry. For example, a nucleic acid encoding one or both of the heavy and light chain polypeptides of a bispecific antibody can be inserted into an expression vector that contains transcriptional and translational regulatory sequences, which include, e.g., promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, transcription terminator signals, polyadenylation signals, and enhancer or activator sequences. The regulatory
sequences include a promoter and transcriptional start and stop sequences. In addition, the expression vector can include more than one replication system such that it can be maintained in two different organisms, for example in mammalian or insect cells for expression and in a prokaryotic host for cloning and amplification.

Several possible vector systems are available for the expression of cloned heavy chain and light chain polypeptides from nucleic acids 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).

The expression vectors can be introduced into cells in a manner suitable for subsequent expression of the nucleic acid. The method of introduction is largely dictated by the targeted cell type, discussed below. Exemplary methods include CaPO₄ precipitation, liposome fusion, lipofectin, electroporation, viral infection, dextran-mediated transfection, polybrene-mediated transfection, protoplast fusion, and direct microinjection.

Appropriate host cells for the expression of bispecific antibodies include yeast, bacteria, insect, plant, and mammalian cells. Of particular interest are bacteria such as E. coli, fungi such as Saccharomyces cerevisiae and Pichia pastoris, insect cells such as SF9, mammalian cell lines (e.g., human cell lines), as well as primary cell lines (e.g., primary mammalian cells).

In some embodiments, a bispecific antibody can be expressed in, and purified from, transgenic animals (e.g., transgenic mammals). For example, a bispecific antibody that binds to C5a and C5b can be produced in transgenic non-human mammals (e.g., rodents, sheep or goats) and isolated from milk as described in, e.g.,

The bispecific antibodies described herein can be produced from cells by culturing a host cell transformed with the expression vector containing nucleic acid encoding the antibodies, under conditions, and for an amount of time, sufficient to allow expression of the proteins. Such conditions for protein expression will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation. For example, antibodies expressed in E. coli can be refolded from inclusion bodies (see, e.g., Hou et al. (1998) Cytokine Jj:3 19-30). Bacterial expression systems and methods for their use are well known in the art (see Current Protocols in Molecular Biology, Wiley & Sons, and Molecular Cloning - A Laboratory Manual - 3rd Ed., Cold Spring Harbor Laboratory Press, New York (2001)). The choice of codons, suitable expression vectors and suitable host cells will vary depending on a number of factors, and may be easily optimized as needed. A bispecific antibody described herein can be expressed in mammalian cells or in other expression systems including but not limited to yeast, baculovirus, and in vitro expression systems (see, e.g., Kaszubska et al. (2000) Protein Expression and Purification 18:213-220).

Following expression, the bispecific antibodies can be isolated. The term "purified" or "isolated" as applied to any of the proteins described herein (e.g., a bispecific antibody) refers to a polypeptide that has been separated or purified from components (e.g., proteins or other naturally-occurring biological or organic molecules) which naturally accompany it, e.g., other proteins, lipids, and nucleic acid in a prokaryote expressing the proteins. Typically, a polypeptide is purified when it constitutes at least 60 (e.g., at least 65, 70, 75, 80, 85, 90, 92, 95, 97, or 99) %, by weight, of the total protein in a sample.

A bispecific antibody can be isolated or purified in a variety of ways known to those skilled in the art depending on what other components are present in the sample. Standard purification methods include electrophoretic, molecular, immunological, and chromatographic techniques, including ion exchange, hydrophobic, affinity, and reverse-phase HPLC chromatography. For example, a bispecific antibody that binds to C5a and C5aR can be purified using a standard anti-antibody column or, e.g., a
protein-A or protein-G column. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. See, e.g., Scopes (1994) "Protein Purification, 3rd edition," Springer-Verlag, New York City, New York. The degree of purification necessary will vary depending on the desired use. In some instances, no purification of the expressed antibody thereof will be necessary.

Methods for determining the yield or purity of a purified antibody are known in the art and include, e.g., Bradford assay, UV spectroscopy, Biuret protein assay, Lowry protein assay, amido black protein assay, high pressure liquid chromatography (HPLC), mass spectrometry (MS), and gel electrophoretic methods (e.g., using a protein stain such as Coomassie Blue or colloidal silver stain).

In some embodiments, endotoxin can be removed from the bispecific antibodies preparations. Methods for removing endotoxin from a protein sample are known in the art and exemplified in the working examples. For example, endotoxin can be removed from a protein sample using a variety of commercially available reagents including, without limitation, the ProteoSpin™ Endotoxin Removal Kits (Norgen Biotek Corporation), Detoxi-Gel Endotoxin Removal Gel (Thermo Scientific; Pierce Protein Research Products), MiraCLEAN® Endotoxin Removal Kit (Minis), or Acrodisc™ - Mustang® E membrane (Pall Corporation).

Methods for detecting and/or measuring the amount of endotoxin present in a sample (both before and after purification) are known in the art and commercial kits are available. For example, the concentration of endotoxin in a protein sample can be determined using the QCL-1000 Chromogenic kit (BioWhittaker), the limulus amebocyte lysate (LAL)-based kits such as the Pyrotell®, Pyrotell®-T, Pyrochrome®, Chromo-LAL, and CSE kits available from the Associates of Cape Cod Incorporated.

Modification of the Bispecific Antibodies

The bispecific antibodies can be modified following their expression and purification. The modifications can be covalent or non-covalent modifications. Such modifications can be introduced into the bispecific antibodies by, e.g., reacting targeted amino acid residues of the polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. Suitable sites for
modification can be chosen using any of a variety of criteria including, e.g., structural analysis or amino acid sequence analysis of the bispecific antibodies.

In some embodiments, the bispecific antibodies can be conjugated to a heterologous moiety. The heterologous moiety can be, e.g., a heterologous polypeptide, a therapeutic agent (e.g., a toxin or a drug), or a detectable label such as, but not limited to, a radioactive label, an enzymatic label, a fluorescent label, or a luminescent label. Suitable heterologous polypeptides include, e.g., an antigenic tag (e.g., FLAG, polyhistidine, hemagglutinin (HA), glutathione-S-transferase (GST), or maltose-binding protein (MBP)) for use in purifying the antibodies. Heterologous polypeptides also include polypeptides that are useful as diagnostic or detectable markers, for example, luciferase, green fluorescent protein (GFP), or chloramphenicol acetyl transferase (CAT). Where the heterologous moiety is a polypeptide, the moiety can be incorporated into a bispecific antibody described herein as a fusion protein.

Suitable radioactive labels include, e.g., $^{32}$P, $^{33}$P, $^{14}$C, $^{125}$I, $^{131}$I, $^{35}$S, and $^{3}$H. Suitable fluorescent labels include, without limitation, fluorescein, fluorescein isothiocyanate (FITC), green fluorescence protein (GFP), DyLight 488, phycoerythrin (PE), propidium iodide (PI), PerCP, PE-Alexa Fluor® 700, Cy5, allophycocyanin, and Cy7. Luminescent labels include, e.g., any of a variety of luminescent lanthanide (e.g., europium or terbium) chelates. For example, suitable europium chelates include the europium chelate of diethylene triamine pentaacetic acid (DTPA) or tetraazacyclododecene-1,4,7,10-tetraacetic acid (DOTA). Enzymatic labels include, e.g., alkaline phosphatase, CAT, luciferase, and horseradish peroxidase.

Two proteins (e.g., a bispecific antibody and a heterologous moiety) can be cross-linked using any of a number of known chemical cross linkers. Examples of such cross linkers are those which link two amino acid residues via a linkage that includes a "hindered" disulfide bond. In these linkages, a disulfide bond within the cross-linking unit is protected (by hindering groups on either side of the disulfide bond) from reduction by the action, for example, of reduced glutathione or the enzyme disulfide reductase. One suitable reagent, 4-succinimidyloloxycarbonyl- α-methyl-α (2-pyridylthio) toluene (SMPT), forms such a linkage between two proteins utilizing a terminal lysine on one of the proteins and a terminal cysteine on the other. Heterobifunctional reagents that cross-link by a different coupling cysteine on each protein can also be used. Other useful cross-linkers include, without
limitation, reagents which link two amino groups (e.g., N-5-azido-2-nitrobenzoyloxy succinimide), two sulfhydryl groups (e.g., 1,4-bis-maleimidobutane), an amino group and a sulfhydryl group (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester), an amino group and a carboxyl group (e.g., 4-[p-azidosalicylamido]butylamine), and an amino group and a guanidinium group that is present in the side chain of arginine (e.g., p-azidophenyl glyoxal monohydrate).

In some embodiments, a radioactive label can be directly conjugated to the amino acid backbone of the bispecific antibody. Alternatively, the radioactive label can be included as part of a larger molecule (e.g., $^{125}$I in meta-$^{125}$Iiodophenyl-N-hydroxysuccinimide ([$^{125}$I]mIPNHS) which binds to free amino groups to form meta-iodophenyl (mIP) derivatives of relevant proteins (see, e.g., Rogers et al. (1997) J. Nucl Med. 38:1221-1229) or chelate (e.g., to DOTA or DTPA) which is in turn bound to the protein backbone. Methods of conjugating the radioactive labels or larger molecules/chelates containing them to the bispecific antibodies described herein are known in the art. Such methods involve incubating the proteins with the radioactive label under conditions (e.g., pH, salt concentration, and/or temperature) that facilitate binding of the radioactive label or chelate to the protein (see, e.g., U.S. Patent No. 6,001,329).

Methods for conjugating a fluorescent label (sometimes referred to as a "fluorophore") to a protein (e.g., a bispecific antibody) are known in the art of protein chemistry. For example, fluorophores can be conjugated to free amino groups (e.g., of lysines) or sulfhydryl groups (e.g., cysteines) of proteins using succinimidy (NHS) ester or tetrafluorophenyl (TFP) ester moieties attached to the fluorophores. In some embodiments, the fluorophores can be conjugated to a heterobifunctional cross-linker moiety such as sulfo-SMCC. Suitable conjugation methods involve incubating a bispecific antibody protein with the fluorophore under conditions that facilitate binding of the fluorophore to the protein. See, e.g., Welch and Redvanly (2003) "Handbook of Radiopharmaceuticals: Radiochemistry and Applications," John Wiley and Sons (ISBN 0471495603).

In some embodiments, the bispecific antibodies can be modified, e.g., with a moiety that improves the stabilization and/or retention of the antibodies in circulation, e.g., in blood, serum, or other tissues. For example, the bispecific antibody can be PEGylated as described in, e.g., Lee et al. (1999) Bioconj. Chem 10(6): 973-8;
Kinstler et al. (2002) *Advanced Drug Deliveries Reviews* 54:477-485; and Roberts et al. (2002) *Advanced Drug Delivery Reviews* 54:459-476. 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.

In some embodiments, the bispecific antibodies described herein can be glycosylated. In some embodiments, a bispecific antibody described herein can be subjected to enzymatic or chemical treatment, or produced from a cell, such that the antibody has reduced or absent glycosylation. Methods for producing antibodies with reduced glycosylation are known in the art and described in, e.g., U.S. patent no. 6,933,368; Wright et al. (1991) *EMBO J* **10**(1):2717-2723; and Co et al. (1993) *Mol Immunol* **30**:1361.

**Pharmaceutical Compositions**

Compositions containing a bispecific antibody described herein can be formulated as a pharmaceutical composition, e.g., for administration to a subject for the treatment or prevention of a complement-associated disorder. 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 (e.g., 4°C). In some embodiments, a composition can be formulated for storage at a temperature below 0°C (e.g., -20°C or -80°C). In some embodiments, the
composition can be formulated for storage for up to 2 years (e.g., one month, two
months, three months, four months, five months, six months, seven months, eight
months, nine months, 10 months, 11 months, 1 year, 1.5 years, or 2 years) at 2-8°C (e.g., 4°C). Thus, in some embodiments, the compositions described herein are stable in storage for at least 1 year at 2-8°C (e.g., 4°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 a bispecific 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 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 an antibody described herein plus any additional desired ingredient (see below) 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, an antibody described herein 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. 7,1 12,341 and 6,019,968; and PCT Publication Nos. WO 00/061 178 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.

Nucleic acids encoding an antibody 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 (see below). Expression constructs of such components may be administered in any therapeutically 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

In some embodiments, a bispecific antibody described herein can be formulated with one or more additional active agents useful for treating or preventing a complement-associated disorder (e.g., an AP-associated disorder or a CP-associated disorder) in a subject. Additional agents for treating a complement-associated disorder in a subject will vary depending on the particular disorder being treated, but can include, without limitation, an antihypertensive (e.g., an angiotensin-converting enzyme inhibitor) [for use in treating, e.g., HELLP syndrome], an anticoagulant, a corticosteroid (e.g., prednisone), or an immunosuppressive agent (e.g., vincristine or cyclosporine A). Examples of anticoagulants include, e.g., warfarin (Coumadin), heparin, phenindione, fondaparinux, idraparinux, and thrombin inhibitors (e.g.,
argatroban, lepirudin, bivalirudin, or dabigatran). A bispecific antibody described herein can also be formulated with a fibrinolytic agent (e.g., ancrod, ε-aminocaproic acid, antiplasmin-ai, prostacyclin, and defibrotide) for the treatment of a complement-associated disorder. In some embodiments, a bispecific antibody can be formulated with a lipid-lowering agent such as an inhibitor of hydroxymethylglutaryl CoA reductase. In some embodiments, a bispecific antibody can be formulated with, or for use with, an anti-CD20 agent such as rituximab (Rituxan™; Biogen Idec, Cambridge, MA). In some embodiments, e.g., for the treatment of RA, the bispecific antibody can be formulated with one or both of infliximab (Remicade®; Centocor, Inc.) and methotrexate (Rheumatrex®, Trexall®). In some embodiments, a bispecific antibody described herein can be formulated with a non-steroidal anti-inflammatory drug (NSAID). Many different NSAIDS are available, some over the counter including ibuprofen (Advil®, Motrin®, Nuprin®) and naproxen (Alleve®) and many others are available by prescription including meloxicam (Mobic®), etodolac (Lodine®), nabumetone (Relafen®), sulindac (Clinoril®), tolementin (Tolectin®), choline magnesium salicylate (Trilasate®), diclofenac (Cataflam®, Voltaren®, Arthrotec®), Diflusinal (Dolobid®), indomethicin (Indocin®), Ketoprofen (Orudis®, Orovail®), Oxaprozin (Daypro®), and piroxicam (Feldene®). In some embodiments a bispecific antibody can be formulated for use with an anti-hypertensive, an anti-seizure agent (e.g., magnesium sulfate), or an anti-thrombotic agent. Anti-hypertensives include, e.g., labetalol, hydralazine, nifedipine, calcium channel antagonists, nitroglycerin, or sodium nitroprussiate. (See, e.g., Mihu et al. (2007) J Gasrtointestinal Liver Dis 16(4):419-424.) Anti-thrombotic agents include, e.g., heparin, antithrombin, prostacyclin, or low dose aspirin.

In some embodiments, a bispecific antibody described herein can be formulated for administration to a subject along with intravenous gamma globulin therapy (IVIG), plasmapheresis, plasma replacement, or plasma exchange. In some embodiments, a bispecific antibody can be formulated for use before, during, or after, a kidney transplant.

When a bispecific antibody is to be used in combination with a second active agent, 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 a bispecific antibody described herein. In some embodiments, a composition can be formulated to include a sub-therapeutic amount of a bispecific antibody and a sub-therapeutic amount of one or more additional active agents such that the components in total are therapeutically effective for treating or preventing a complement-associated disorder (e.g., an alternative complement pathway-associated complement disorder or a classical complement pathway-associated disorder). In some embodiments, a composition can be formulated to include, e.g., a first bispecific antibody that binds to C5aR and C5a and a second bispecific antibody that binds to C5b and C5a in accordance with the disclosure, each at a sub-therapeutic dose, such that the antibodies in total are at a concentration that is therapeutically effective for treating a complement-associated disorder. Methods for determining a therapeutically effective dose of an agent such as a therapeutic antibody are known in the art and described herein.

**Methods for Treatment**

The above-described compositions (e.g., any of the bispecific antibodies 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.

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 bispecific 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 antibody that binds to C5a and C5b may be required to treat a subject with RA
as compared to the dose of an antibody that binds to C5a and C5aR1 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
antibody that binds to C5a and C5b 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.

A pharmaceutical composition can include a therapeutically effective amount
of an 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 any of the bispecific antibodies described herein can
further be evaluated in, e.g., Phase I dose escalation studies. See, e.g., van Gurp et al.
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 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
antibody, which specifically binds to C5a and C5b. In some embodiments, a
composition described herein contains a therapeutically effective amount of an
antibody, which specifically binds to C5a and C5aRI. In some embodiments, a
composition described herein contains a therapeutically effective amount of an
antibody, which specifically binds to C5b and C5aR. In some embodiments, a
composition described herein contains a therapeutically effective amount of an
antibody that specifically binds to: (i) C5a or a cellular receptor for C5a and (ii) a
component or intermediate of the TCC including, e.g., C5b-6, C5b-7, C5b-8, or C5b-9.
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 a bispecific 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 bispecific antibody (e.g., an antibody that binds to C5a and C5b, an antibody that binds to C5a and C5aR, an antibody that binds to C5b and C5aR, an antibody that binds to C5a and a component or intermediate of the TCC) 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 antibodies lies generally within a range of circulating concentrations of the bispecific antibodies 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 bispecific 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 replacement, or plasma exchange. See, e.g., Appel et al. (2005) JAm. Soc Nephrol. J5: 1392-1404. In some embodiments, a bispecific 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.

A "subject," as used herein, can be any mammal. For example, a subject can be a human, 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).

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 an antibody that binds to C5a and C5b, an antibody that binds to the C5a and C5aR, or an antibody that binds to C5b and C5aR.

As described above, the bispecific antibodies 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; asthma; ischemia-reperfusion injury; atypical hemolytic uremic syndrome (aHUS); typical or infectious hemolytic uremic syndrome (tHUS); dense deposit disease (DDD); paroxysmal nocturnal hemoglobinuria (PNH); neuromyelitis optica (NMO); multifocal motor neuropathy (MMN); multiple sclerosis (MS); Degos' disease; 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, but not limited to, a cardiovascular disorder, myocarditis, a cerebrovascular disorder, a peripheral (e.g., musculoskeletal) vascular disorder, a renovascular disorder, a mesenteric/enteric vascular disorder, revascularization to transplants and/or replants, 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, Guillain-Barre Syndrome, graft rejection (e.g., transplant rejection, e.g., kidney, liver, heart, bone marrow, or skin transplant rejection), systemic inflammatory response sepsis, septic shock, spinal cord injury, glomerulonephritis, Hashimoto's thyroiditis, type I diabetes, psoriasis, pemphigus, AIHA, ITP, Goodpasture syndrome, 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 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.
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 ADAMTS 13 gene. ADAMTS 13 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. 16:177-183. 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 ADAMTS 13 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 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 121:940-943; 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)
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 Kaser 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., Soltesz et al. (2000) Haematologica (Budap) 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) Haematologica 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 ADAMTS 13 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* 162:31 1-316; and Padden (1999), *supra.*) Symptoms of PNH include, e.g., hemolytic anemia (a decreased number of red blood cells), hemoglobinuria (the presence of hemoglobin in the urine particularly evident after sleeping), and hemoglobinemia (the presence of hemoglobin in the bloodstream). PNH-affected subjects are known to have paroxysms, which are defined here as incidences of dark-colored urine, dysphagia, fatigue, erectile dysfunction, thrombosis, and recurrent abdominal pain.

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 e t al. (2006), supra; Ault e t al. (1997) J Biol. Chem. 272:25168-75; Abrera-Abeleda e t 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
ADAMTS 13) 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 ADAMTS 13 activity assays to determine if an anti-ADAMTS13 antibody is present. See, e.g., Dong et al. (2008) Am JHematol. 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 ADAMTS 13 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 thrombopoiesis. 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

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 NYAcad 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).

Methods for identifying a subject as having, suspected of having, or at risk for developing PNH are known in the art of medicine. The laboratory evaluation of hemolysis normally includes hematologic, serologic, and urine tests. Hematologic tests include an examination of the blood smear for morphologic abnormalities of red blood cells (RBC), and the measurement of the reticulocyte count in whole blood (to determine bone marrow compensation for RBC loss). Serologic tests include lactate dehydrogenase (LDH; widely performed), and free hemoglobin (not widely performed) as a direct measure of hemolysis. LDH levels, in the absence of tissue damage in other organs, can be useful in the diagnosis and monitoring of patients with hemolysis. Other serologic tests include bilirubin or haptoglobin, as measures of breakdown products or scavenging reserve, respectively. Urine tests include bilirubin, hemosiderin, and free hemoglobin, and are generally used to measure gross severity of hemolysis and for differentiation of intravascular vs. extravascular etiologies of hemolysis rather than routine monitoring of hemolysis. Further, RBC numbers, RBC hemoglobin, and hematocrit are generally performed to determine the extent of any accompanying anemia.

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 striatrial 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. 177:1 19-131; 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 230^: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.

In some embodiments, a bispecific 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, PNH, RA, HELLP, MG, CAD, CAPS, tHUS, or any other complement-associated disorder known in the art or 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 bispecific antibody and an immunosuppressive agent such as Remicade® for use in treating RA. In some embodiments, the bispecific antibody and the one or more additional active agents are administered at the same time. In other embodiments, a bispecific antibody 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 bispecific antibody is administered second in time.

A bispecific antibody described herein can replace or augment a previously or currently administered therapy. For example, upon treating with an antibody that binds to C5a and C5b, 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 bispecific antibody reaches a
level sufficient to provide a therapeutic effect. The two therapies can be administered in combination.

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 known in the art and/or 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.

**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 bispecific antibody 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 C5a and C5b, or the cells can be transduced 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 bispecific antibody 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 bispecific

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 bispecific antibodies. For example, the kit can contain one or more of any of the bispecific antibodies described herein. 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, but not limited to, DDD, aHUS, TTP, HELLP, RA, PNH, AMD, tHUS, MG, CAD, CAPS, or any other complement pathway-associated disorder known in the art and/or 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. Treatment of thrombotic thrombocytopenic purpura using a bispecific antibody**

A human patient is identified by a medical practitioner as having an inherited form of TTP. Once a week for four weeks the patient is administered a composition containing a bispecific antibody that binds to C5a and C5b by intravenous infusion. The patient and medical practitioner observe a substantial improvement in at least two known symptoms of TTP during the initial treatment. One week after the initial four week treatment, the patient receives intravenously administered "maintenance doses" of the antibody every two weeks until the medical practitioner determines that the TTP is in remission.

**Example 2. Treatment of dense deposit disease using a bispecific antibody**

A human patient presenting with DDD is intravenously administered every two weeks a composition containing a bispecific antibody that binds to C5aR1 and C5b. The patient and medical practitioner observe a substantial reduction in overall severity of the patient's DDD symptoms during the initial treatment. The patient is maintained on the same treatment regimen until the medical practitioner determines that the DDD is in remission.

**Example 3. Effect of human C5 on the clearance of a humanized anti-C5 antibody in mice**

The following experiments were performed to determine the effect of human C5 on the clearance of a humanized anti-C5 antibody in a humanized neonatal Fc receptor (hFcRn) mouse model which is lacking endogenous FcRn and is transgenic for hFcRn (niFcRnV hFcRn +/+; Jackson Laboratories, Bar Harbor, Maine). The humanized FcRn model has been described in, e.g., Petkova et al. (2006) *Int Immunology* 18(12): 1759-1769 and Otao et al. (2008) *Proc Natl Acad Sci USA* 105(27):9337-9342. 100 µg of a humanized anti-human C5 antibody in 200 µL of phosphate buffered saline (PBS) was administered by intravenous (i.v.) injection to each of eight (8) hFcRn transgenic mice. Serum was collected from each of the mice at days one, three, seven, 14, 21, 28, and 35 following the administration. The
concentration of the humanized antibody in the serum was measured by ELISA. Briefly, assay plates were coated with an anti-human K light chain capture antibody followed by washing to remove unbound capture antibody. The wells of the plate were then contacted with the serum samples under conditions that allow the humanized anti-human C5 antibody, if present in the serum, to bind to the capture antibody. The relative amount of humanized antibody bound to each well was detected using a detectably-labeled anti-human IgG antibody.

The half-life of the antibody in the mice was calculated using the ELISA measurements and the following equation (where $T$ is the time evaluated, $A_0$ is the initial concentration of the antibody, and $A_t$ is the concentration of the antibody in serum determined at time $T$).

$$\text{Half-life (T}_{1/2} = T \times \frac{(\ln 2)}{\ln(A_0/A_t)} \quad \text{(Equation 1)}$$

The results of the experiment are depicted in Fig. 1. The half-life of the humanized anti-C5 antibody in the hFcRn mouse model was $12.56 \pm 1.73$ days.

To determine the effect of human C5 on the half-life of the humanized antibody using the hFcRn model, mice were administered in 250 µL PBS one of: (i) 50 µg of the humanized antibody complexed in a 1:4 molar ratio of antibody to human C5 (6 mice); (ii) 50 µg of the humanized antibody complexed in a 1:4 molar ratio of antibody to human C5 and an additional 200 µg of human C5 (in 200 µL of PBS) by i.v. injection (6 mice); (iii) 50 µg of the humanized antibody complexed in a 1:2 molar ratio of antibody to human C5 (6 mice); or (iv) 50 µg of the humanized antibody alone (6 mice). Serum was collected from the mice, as described above, at days one, three, seven, 14, 21, 28, and 35 following the administration and the half-life of the humanized antibody under each condition was determined as described above.

The half-life of the humanized anti-human C5 antibody, in the absence of human C5, was determined in this experiment to be $13.49 \pm 0.93$ days. In contrast, the half-life of the humanized antibody administered to the mice in a 1:2 ratio with human C5 was measured to be $9.58 \pm 1.24$ days. The half-life of the humanized antibody administered to the mice in a 1:4 ratio with human C5 was determined to be
5.77 ± 1.86 days. The additional administration of human C5 along with the 1:4 antibody-C5 complex to mice resulted in a half-life for the antibody of 4.55 ± 1.02 days.

These results indicate that the clearance of the humanized anti-C5 antibody in this mouse model is greatly influenced by the concentration of its antigen. In other words, the half-life of the antibody in this model is dependent on the amount of uncomplexed antibody. Human C5 is constitutively expressed and present in serum at a concentration of approximately 0.37 μM. Unlike C5, fragments C5a and C5b are present in blood at much lower concentrations and are often restricted to specific areas of local complement activation. The data presented herein indicate that a lower concentration of fragments C5a and C5b, as compared to C5, will favor a longer half-life for a bispecific antibody (e.g., an anti-C5a/C5b antibody) over an anti-C5 antibody in blood due to a reduced contribution of target-mediated antibody clearance. The data also indicate that a lower dose and/or lower frequency administration of an anti-C5a/C5b bispecific antibody, as compared to an anti-C5 antibody, can provide the same or greater inhibition of C5 in a human with a complement-associated disorder such as PNH or aHUS.

Example 4. Mathematical modeling of the clearance of a humanized anti-C5 antibody

Example 3 demonstrated that the presence of an excess of C5 over antibody leads to an approximate three-fold reduction in the half life of an anti-C5 monoclonal antibody. A simple mathematical modeling featuring target (C5) mediated clearance was developed and used to explore the potential mechanism behind this effect. The model that invokes the basic pathways of clearance of the humanized antibody in a human patient is shown in Fig. 2. Free antibody (A) and its antigen C5 (C) are in equilibrium with their cognate, complexed form. The rate constant for association of the antibody and C5 is represented by k3 and the rate constant for the dissociation of the complex is represented by k4. The antibody:C5 complex (CA) can be eliminated by immune complex clearance with a rate constant represented by k6. Free antibody is also eliminated as represented by a different rate constant k5. C5 is constitutively expressed with a rate constant of k1 and it is eliminated with a rate constant of k2.
The model was also based on a series of assumptions. First, the rate of C5 synthesis (rate constant $k_l$) is constant and therefore zero order and in the presence of excess antibody the clearance of free C5 via rate constant $k_l$ is negligible because the concentration of free C5 is negligible. In addition, the clearance of free antibody is assumed to be controlled by first order beta-elimination. Also assumed was that because the antibody:C5 complex does not accumulate, the rate of synthesis of C5 is limiting for the rate of complex elimination (via rate constant $k_\ell$). Lastly, the model assumes that no free antibody is recycled from the immune complex. In other words, the complex is either dissociated or eliminated - it is assumed that the immune complex-mediated clearance (rate constant $k_\ell$) is irreversible.

Based on these assumptions, a simplified pathway of antibody clearance was constructed (Fig. 3). Like the pathway depicted in Fig. 2, the simplified pathway consists of two modes of antibody clearance: (i) free antibody clearance with rate constant $k_8$ and (ii) immune complex clearance with rate constant $k_l$. The first order equation that governs the free antibody clearance is as follows (where $A$ is the concentration of antibody at the time measured, $A_0$ is the initial concentration of antibody, and $t$ is the time at which $A$ is measured).

$$A = A_0 \times e^{-k_8 t}$$  
(Equation 2)
The zero order equation governing the immune complex clearance is represented by
the following equation.

\[ A = A_0 - kit \]  
(Equation 3)

The integrated rate equation for the concurrent processes was determined to be

\[ A = A_0 \times e^{-kit}. \]  
(Equation 4)

It was assumed that \( A_0 \), the initial antibody concentration, is 400 \( \mu \)g/mL or
2.67 \( \mu \)M for a 150 kDa antibody. The physiological concentration of human C5 is
0.37 \( \mu \)M for a 190 kDa protein. One antibody has two antigen-combining sites and
therefore binds to two C5 molecules. Breakthrough is obtained when the
concentration of the free antibody is zero. See, e.g., Brodsky et al. (2008) Blood

As C5 is constitutively expressed and maintained at a constant concentration
in the blood, the rate of synthesis of C5 protein is equal to its rate of clearance. The
half-life of human C5 in blood is approximately 63 hours (see, e.g., Sissons et al.
(1977) J Clin Invest 59:704-715). Thus, the rate constant, \( kl \), for C5 clearance or
production is approximately 0.185 \( \mu \)M/ (63 ÷ 24) day\(^{-1}\) or 0.07 \( \mu \)M day\(^{-1}\).

To determine the rate constant \( k8 \) for the first order antibody clearance
(Equation 2), the relationship between the rate constant and the half-life can be
represented by the following equation:

\[ T_y = \ln2/k8. \]  
(Equation 5)

It was assumed that the half life of the anti-C5 monoclonal antibody in the absence of
C5 is the same as the value (12.56 days) obtained in FcRn mice (see Example 3).
Thus, solving Equation 5 for \( k8 \), \( k8 \) is equal to In2/Ty or (0.693)/(12.56 days) or 0.055
day\(^{-1}\). The calculated influence of immune complex clearance on the half-life of a
humanized anti-C5 antibody is set forth in Table 1.
Table 1.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Time (days)</th>
<th>$A_0 \times e^{-kt}$</th>
<th>$-kt$</th>
<th>$A$</th>
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<tr>
<td>Anti-C5 Ab</td>
<td>0</td>
<td>2.67</td>
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<td></td>
<td>5</td>
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<td>-0.35</td>
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<td></td>
<td>15</td>
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<td>-1.05</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>1.11</td>
<td>1.12</td>
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</tr>
</tbody>
</table>

From Table 1, breakthrough is achieved by 16 days. In other words, the levels of an antibody with a half-life of 12.56 days is reduced to zero by 16 days through the effects of the C5 mediated clearance component, whereas in theory approximately 40% of the starting concentration of this antibody would have remained without the effects of target (C5) mediated clearance component. In fact, the rate of clearance predicted by the above-described model closely overlaps with the rate observed in vivo. Based on the model and the calculations described above, the contribution of target-mediated clearance (immune complex clearance) on the half-life of the humanized antibody in man is substantial. This model, as well as the in vivo data, strongly indicates that a lower concentration of fragments C5a and C5b, as compared to C5, will favor a longer half-life for a bispecific antibody (e.g., an anti-C5a/C5b antibody) over an anti-C5 antibody in blood due to a reduced contribution of target-mediated antibody clearance. Accordingly, a lower dose and/or lower frequency administration of an anti-C5a/C5b bispecific antibody, as compared to an anti-C5 antibody, is likely to provide the same or greater inhibition of C5 in a human with a complement-associated disorder.

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 bispecific antibody that binds to: (a) C5a and C5aRl; (b) C5a and C5b; (c) C5b and C5aRl; (d) C5a and C5L2; (e) C5b and C5L2; (f) C5aRl and C5L2; (g) C5b-6 and C5a; (h) C5b-6 and C5b; (i) C5b-6 and C5aRl; or (j) C5b-6 and C5L2.

2. A bispecific antibody comprising at least two different antigen combining sites, wherein at least one antigen combining site binds to C5a and at least one antigen combining site binds to C5b or C5aR.

3. A bispecific antibody comprising at least two different antigen combining sites, wherein at least one antigen combining site binds to C5b and at least one antigen combining site binds to C5aR.

4. A bispecific antibody comprising at least two different antigen combining sites, wherein
   (i) at least one antigen combining site binds to C5a or C5b; and
   (ii) at least one antigen combining site binds to a cellular receptor for C5a.

5. A bispecific antibody comprising at least two different antigen combining sites, wherein
   (i) at least one antigen combining site binds to C5a, C5b, or a cellular receptor for C5a; and
   (ii) at least one antigen combining site binds to C5b-6.

6. The bispecific antibody of claim 4 or 5, wherein the cellular receptor is C5aRl.

7. The bispecific antibody of claim 4 or 5, wherein the cellular receptor is C5L2.

8. A bispecific antibody having binding specificity for at least two of C5a, C5aRl, C5b, C5L2, C5b-6, C5b-7, C5b-8, and C5b-9.

9. The bispecific antibody of any one of claims 1-8, wherein the antibody does not bind to full length C5.
10. The bispecific antibody of any one of claims 1 or 5-9, wherein the antibody does not bind to uncomplexed C5b or to uncomplexed C6.

11. A bispecific antibody comprising:
   (i) a first antigen combining site that binds to C5a; and
   (ii) a second antigen combining site that binds to a cellular receptor for C5a.

12. The bispecific antibody of claim 11, wherein the first antigen combining site binds to desarginated C5a.

13. The bispecific antibody of claim 11 or 12, wherein the first antigen combining site binds to a mammalian C5a.

14. The bispecific antibody of claim 13, wherein the mammalian C5a is human C5a.

15. The bispecific antibody of claim 11, wherein the first antigen combining site binds to a C5a protein having an amino acid sequence that is at least 90% identical to the amino acid sequence depicted in SEQ ID NO:1.

16. The bispecific antibody of claim 11, wherein the first antigen combining site binds to a fragment of a human C5a protein comprising the amino acid sequence depicted in any one of SEQ ID NOs:2-14.

17. The bispecific antibody of claim 11, wherein the first antigen combining site binds to an epitope comprising at least 4 consecutive amino acids depicted in any one of SEQ ID NOs:1-14.

18. The bispecific antibody of any one of claims 11-17, wherein the cellular receptor is C5aR1.

19. The bispecific antibody of claim 18, wherein C5aR1 is a mammalian C5aR1.
20. The bispecific antibody of claim 19, wherein the mammalian C5aR1 is human C5aR1.

21. The bispecific antibody of claim 18, wherein the second antigen combining site binds to a C5aR1 protein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence depicted in SEQ ID NO: 17.

22. The bispecific antibody of claim 18, wherein the second antigen combining site binds to a fragment of a human C5aR1 protein comprising the amino acid sequence depicted in any one of SEQ ID NOs: 18-22.

23. The bispecific antibody of claim 18, wherein the second antigen combining site binds to an epitope comprising at least 4 consecutive amino acids depicted in any one of SEQ ID NOs: 17-22.

24. The bispecific antibody of any one of claims 11-17, wherein the cellular receptor is C5L2.

25. The bispecific antibody of any one of claims 24, wherein C5L2 is a mammalian C5L2.

26. The bispecific antibody of claim 25, wherein the mammalian C5L2 is human C5L2.

27. The bispecific antibody of claim 24, wherein the second antigen combining site binds to a C5L2 protein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence depicted in SEQ ID NO:23.

28. A bispecific antibody comprising:
   (i) a first antigen combining site that binds to C5a; and
   (ii) a second antigen combining site that binds to C5b.
29. The bispecific antibody of claim 28, wherein the first antigen combining site binds to desarginated C5a.

30. The bispecific antibody of claim 28 or 29, wherein the first antigen combining site binds to a mammalian C5a.

31. The bispecific antibody of claim 30, wherein the mammalian C5a is human C5a.

32. The bispecific antibody of claim 28, wherein the first antigen combining site binds to a C5a protein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence depicted in SEQ ID NO:1.

33. The bispecific antibody of claim 28, wherein the first antigen combining site binds to a fragment of a human C5a protein comprising the amino acid sequence depicted in any one of SEQ ID NOs:2-14.

34. The bispecific antibody of claim 28, wherein the first antigen combining site binds to an epitope comprising at least 4 consecutive amino acids depicted in any one of SEQ ID NOs:1-14.

35. The bispecific antibody of any one of claims 28-34, wherein the second antigen combining site binds to a mammalian C5b.

36. The bispecific antibody of claim 35, wherein the mammalian C5b is human C5b.

37. The bispecific antibody of claim 28, wherein the second antigen combining site binds to a C5b protein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence depicted in SEQ ID NO: 15 or 16.

38. The bispecific antibody of claim 28, wherein the second antigen combining site binds to an epitope comprising at least four consecutive amino acids depicted in SEQ ID NO:15 or 16.
39. The bispecific antibody of any one of claims 28-38, wherein the antibody does not bind to full-length C5.

40. A bispecific antibody comprising:
   (i) a first antigen combining site that binds to C5b; and
   (ii) a second antigen combining site that binds to a cellular receptor for C5a.

41. The bispecific antibody of claim 40, wherein the first antigen combining site binds to a mammalian C5b.

42. The bispecific antibody of claim 41, wherein the mammalian C5b is human C5b.

43. The bispecific antibody of claim 40, wherein the first antigen combining site binds to an amino acid sequence that is at least 90% identical to the amino acid sequence depicted in SEQ ID NO: 15 or 16.

44. The bispecific antibody of claim 40, wherein the first antigen combining site binds to an epitope comprising at least four consecutive amino acids depicted in SEQ ID NO:15 or 16.

45. The bispecific antibody of any one of claims 40-44, wherein the cellular receptor is C5aR1.

46. The bispecific antibody of claim 45, wherein the C5aR1 is a mammalian C5aR1.

47. The bispecific antibody of claim 46, wherein the mammalian C5aR1 is human C5aR1.

48. The bispecific antibody of claim 40, wherein the second antigen combining site binds to an amino acid sequence that is at least 90% identical to the amino acid sequence depicted in SEQ ID NO: 17.
49. The bispecific antibody of claim 40, wherein the second antigen combining site binds to a fragment of a human C5aRI protein comprising the amino acid sequence depicted in any one of SEQ ID NOs: 18-22.

50. The bispecific antibody of claim 40, wherein the second antigen combining site binds to an epitope comprising at least 4 consecutive amino acids depicted in any one of SEQ ID NOs: 17-22.

51. The bispecific antibody of any one of claims 40-44, wherein the cellular receptor is C5L2.

52. The bispecific antibody of any one of claims 51, wherein C5L2 is a mammalian C5L2.

53. The bispecific antibody of claim 52, wherein the mammalian C5L2 is human C5L2.

54. The bispecific antibody of claim 40, wherein the second antigen combining site binds to an amino acid sequence that is at least 90% identical to the amino acid sequence depicted in SEQ ID NO:23.

55. The bispecific antibody of claim 40, wherein the second antigen combining site binds to an epitope comprising at least four consecutive amino acids of SEQ ID NO:23.

56. The bispecific antibody of any one of claims 1-55, wherein the antibody inhibits the interaction between C5a and a cellular receptor for C5a.

57. The bispecific antibody of any one of claims 1-10 or 28-55, wherein the antibody inhibits the assembly or activity of the C5b-9 complex.

58. The bispecific antibody of any one of claims 1-57, wherein the antibody inhibits C5a-dependent chemotaxis.
59. The bispecific antibody of any one of claims 1-10 or 28-57, wherein the antibody inhibits the interaction between C5b and C6.

60. The bispecific antibody of any one of claims 1-10 or 28-59, wherein the antibody inhibits complement-dependent lysis in vitro.

61. The bispecific antibody of any one of claims 1-39, wherein the antibody binds to C5a with a $K_a$ of at least $10^8 \text{ M}^{-1}$.

62. The bispecific antibody of any one of claims 1-6, 8, 18-23, or 45-50, wherein the antibody binds to C5aRI with a $K_a$ of at least $10^8 \text{ M}^{-1}$.

63. The bispecific antibody of any one of claims 1-10 or 28-56, wherein the antibody binds to C5b with a $K_a$ of at least $10^8 \text{ M}^{-1}$.

64. The bispecific antibody of any one of claims 1-63, further comprising a third antigen combining site that binds to an antigen present in full-length C5 or a terminal complement protein selected from the group consisting of C6, C7, C8, and C9.

65. The bispecific antibody of any one of claims 1-64, wherein the antibody is a monoclonal antibody.

66. The bispecific antibody of any one of claims 1-64, wherein the antibody is a single-chain antibody.

67. The bispecific antibody of any one of claims 1-66, wherein the antibody is a humanized antibody.

68. The bispecific antibody of any one of claims 1-64, wherein the antibody comprises two different monospecific antibodies that are associated with one another.
69. The bispecific antibody of any one of claims 1-64, wherein the antibody is selected from the group consisting of a recombinant antibody, a diabody, an intrabody, a chimerized or chimeric antibody, a deimmunized human antibody, a fully human antibody, and an F(ab')\textsubscript{2} fragment.

70. The bispecific antibody of any one of claims 1-64, wherein the antibody is a single chain diabody, a tandem single chain Fv fragment, a tandem single chain diabody, or a fusion protein comprising a single chain diabody and at least a portion of an immunoglobulin heavy chain constant region.

71. The bispecific antibody of any one of claims 1-64, wherein the antibody is a dual variable domain immunoglobulin.

72. The bispecific antibody of any one of claims 1-64, wherein the antibody comprises a heterologous moiety.

73. The bispecific antibody of claim 72, wherein the heterologous moiety is a sugar.

74. The bispecific antibody of claim 73, wherein the antibody is glycosylated.

75. The bispecific antibody of claim 74, wherein the heterologous moiety is a detectable label.

76. The bispecific antibody of claim 75, wherein the detectable label is a fluorescent label, a luminescent label, a heavy metal label, a radioactive label, or an enzymatic label.

77. The bispecific antibody of claim 76, wherein the fluorescent label is selected from the group consisting of fluorescein, fluorescein isothiocyanate (FITC), green fluorescent protein (GFP), DyLight 488, phycoerythrin (PE), propidium iodide (PI), PerCP, PE-Alexa Fluor® 700, Cy5, allophycocyanin, and Cy7.
78. The bispecific antibody of claim 76, wherein the enzymatic label is horseradish peroxidase, alkaline phosphatase, or luciferase.

79. The bispecific antibody of claim 76, wherein the radioactive label is selected from the group consisting of $^{32}$P, $^{33}$P, $^{14}$C, $^{125}$I, $^{131}$I, $^{35}$S, and $^{3}$H.

80. A composition comprising the bispecific antibody of any one of claims 1-79 and a pharmaceutically-acceptable carrier.

81. The composition of claim 80, wherein the composition comprises the bispecific antibody of claim 11.

82. The composition of claim 80, wherein the composition comprises the bispecific antibody of claim 28.

83. The composition of claim 80, wherein the composition comprises the bispecific antibody of claim 40.

84. A method for inhibiting terminal complement in a subject, the method comprising administering to a subject in need thereof an antibody in an amount effective to inhibit terminal complement in the subject, wherein the antibody is the bispecific antibody of any one of claims 1, 3, 4, 5, 8-10, or 28-79.

85. A method for treating a complement-associated disorder in a subject, the method comprising administering to a subject in need thereof an antibody in an amount effective to treat a complement-associated disorder in the subject, wherein the antibody is the bispecific antibody of any one of claims 1-79.

86. The method of claim 85, wherein the complement-associated disorder is an alternative complement pathway-associated disorder.

87. The method of claim 86, wherein the alternative complement pathway-associated disorder is selected from the group consisting of rheumatoid arthritis, asthma,
ischemia-reperfusion injury, atypical hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, paroxysmal nocturnal hemoglobinuria, dense deposit disease, age-related macular degeneration, spontaneous fetal loss, Pauci-immune vasculitis, epidermolysis bullosa, recurrent fetal loss, multiple sclerosis, and traumatic brain injury.

88. The method of claim 85, wherein the complement-associated disorder is a classical complement pathway-associated disorder.

89. The method of claim 88, wherein the classical complement pathway-associated disorder is selected from the group consisting of 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, and catastrophic antiphospholipid syndrome.

90. The method of any one of claims 85-89, further comprising identifying the subject as having, suspected of having, or at risk for developing, a complement-associated disorder.

91. The method of any one of claims 85-90, further comprising, after the administering, monitoring the subject for an improvement in one or more symptoms of the complement-associated disorder.

92. The method of any one of claims 84-91, wherein the antibody is intravenously administered to the subject.

93. The method of any one of claims 84-91, wherein the subject is a mammal.

94. The method of claim 93, wherein the mammal is a human.
Zero order:
\[ A = A_0 - k_7t \]

Immune complex clearance

Integrated rate equation for the concurrent processes:
\[ A = A_0 e^{-k_8 t} - k_7 t \]

1st order
\[ A = A_0 e^{-k_8 t} \]
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US20 10/039448

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.
C07K 16/46 (2006.01) A61P 31/00 (2006.01) C07K 16/18 (2006.01) A61K 39/00 (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)
WPIDS, EPDOC, MEDLINE (Keyword; bispecific, bivalent, bsab, bsmab, trispecific, trivalent, complement 5, C5a, C5b, C5aRI, C5L2 and like terms), EPDOC (ECLA C07K 16/46D, Full text search, Keywords: bivalent, bsab, bsmab, trispecific, trivalent, complement 5, C5a, C5b, C5aRI, C5L2)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

Further documents are listed in the continuation of Box C

See patent family annex

Date of the actual completion of the international search: 23 August 2010
Date of mailing of the international search report: 2 SEP 2010

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(ISO 9001 Quality Certified Service)
Telephone No: +61 2 6283 2747

Form PCT/ISA/210 (second sheet) (July 2009)
1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)
      - [ ] on paper
      - [ ] in electronic form

   b. (time)
      - [ ] in the international application as filed
      - [ ] together with the international application in electronic form
      - [ ] subsequently to this Authority for the purposes of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments;
   A sequence listing was filed but it was not used for the purpose of this search and opinion.
INTERNATIONAL SEARCH REPORT

<table>
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<th>Box No. I</th>
<th>Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)</th>
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<td></td>
<td>This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:</td>
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<tr>
<td>1.</td>
<td>[ ] Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:</td>
</tr>
<tr>
<td>2.</td>
<td>[ ] Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:</td>
</tr>
<tr>
<td>3.</td>
<td>[ ] Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)</td>
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<th>Box No. III</th>
<th>Observations where unity of invention is lacking (Continuation of item 3 of first sheet)</th>
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<tr>
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<td>This International Searching Authority found multiple inventions in this international application, as follows:</td>
</tr>
<tr>
<td></td>
<td>• Invention 1: Claims 1, 2, 4; 8, 9, 56-61, 63-80, 84-94 (partially) and claims 28-39, 82 (fully) are directed to a bispecific antibody that binds to C5a and C5b and its method of use in terminal complement or complement associated disorders. It is considered that a bispecific antibody targeted to C5a and C5b comprises a first distinguishing feature.</td>
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<td>• Invention 2: Claims 1, 2, 4, 6-10, 56, 58, 61, 62, 64-80, 84-94 (partially) and claims 11-27, 81 (fully) are directed to a bispecific antibody that binds to C5a and C5a receptors (C5aR1 and C5L2) and its method of use in terminal complement or complement associated disorders. It is considered that a bispecific antibody and their use, wherein the antibody are targeted to C5a and its receptor comprises a second distinguishing feature.</td>
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<tr>
<td>1.</td>
<td>[ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims</td>
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<tr>
<td>2.</td>
<td>[X] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.</td>
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<tr>
<td>3.</td>
<td>[ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:</td>
</tr>
<tr>
<td>4.</td>
<td>[ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:</td>
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Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/21 0 (continuation of first sheet (2)) (July 2009)
<table>
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<tr>
<td>WO 2010/01 1697 A1 (MMUNOMEDICS, INC.) 28 January 2010</td>
<td>See claim 49</td>
<td>1-94</td>
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<tr>
<td>WO 2006/063 150 A2 (MMUNOMEDICS, INC.) 15 June 2006</td>
<td>See paragraphs [0034], [00170] and claim 17</td>
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**Supplemental Box I**
(To be used when the space in any of Boxes I to IV is not sufficient)

**Continuation of Box No: III** Observations where unity of invention is lacking

- **Invention 3:** Claims 1, 3, 4, 6-9 (partially), 56-60, 62-80, 84-94 and claims 40-55, 83 (fully) are directed to a bispecific antibody that binds to C5b and C5a receptors (C5aR1 and C5L2) and its method of use in terminal complement or complement associated disorders. It is considered that a bispecific antibody and their use, wherein the antibody are targeted to C5b and a C5a receptor comprises a third distinguishing feature.

- **Inventions 4-6:** Claims 1, 5, 8, 9, 10, 56-61, 64-80, 84-94 (partially) are directed to bispecific antibodies that bind to C5a, C5b or C5a receptor; and a C5b complex (C5b-6, C5b-7, C5b-8 and C5b-9) and their method of use in terminal complement or complement associated disorders. It is considered that bispecific antibodies and their use, wherein the antibodies are targeted to C5a, C5b or C5a receptor; and a C5b complex (C5b-6, C5b-7, C5b-8 and C5b-9) comprise the fourth, fifth and sixth distinguishing features respectively.

- **Invention 7:** Claims 8-10 (partially) are directed to bispecific antibodies that bind to at least two of C5b complexes C5b-6, C5b-7, C5b-8 and C5b-9 and their method of use in terminal complement or complement associated disorders. It is considered that bispecific antibodies and their use, wherein the antibodies are targeted to at least two of C5b complexes C5b-6, C5b-7, C5b-8 and C5b-9 comprises the seventh distinguishing feature.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

The only feature common to all of the claims is Bispecific antibodies targeted to C5 fragment C5a, C5 fragment C5b and/or C5 fragment complexes (C5b-6, C5b-7, C5b-8 or C5b-9) of the complement cascade. However this concept is not novel in the light of:

D4 WO 2006/063150 A2. D4 (see paragraphs [0034], [00170] and claim 17) which discloses bispecific antibody capable of interfering with the complement cascade by binding to C5a.

This means that the common feature can not constitute a special technical feature within the meaning of PCT Rule 13.2, second sentence, since it makes no contribution over the prior art.

Because the common feature does not satisfy the requirement for being a special technical feature it follows that it cannot provide the necessary technical relationship between the identified inventions. Therefore the claims do not satisfy the requirement of unity of invention _aposteriori._
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.

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

END OF ANNEX