

Targeted Treatment of Complement-Mediated Disease Through Local Complement
Inhibition Based on Urinary uC5b-9 Detection

REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to and the benefit of the filing date of U.S. Provisional Patent Application No. 63/527,123, filed on July 17, 2023, the entire contents of which are incorporated herein by reference.

SEQUENCE LISTING

[0002] The present application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on July 12, 2024, is named 132301-01020.xml and is 537,649 bytes in size. The sequence listing contained in this .XML file is part of the specification and is hereby incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

[0003] The complement system is a protease cascade of the innate immune system that triggers inflammation, and helps immune cells to fight infections following activation by immune complexes, apoptotic cells or foreign sugar motifs. Thus, complement provides the first line of defense against pathogens, and is a bridge between the innate and adaptive immune systems. The complement cascade can be initiated either through the lectin pathway (LP) by mannose-binding lectins or through the classical pathway (CP) by IgM or IgG clustering. A third arm of the complement cascade, the alternative pathway (AP), is constitutively active at a low level and can amplify CP- and LP-initiated complement activation. Each pathway leads to the cleavage of the central component C3 into bioactive fragments C3a and C3b, ultimately activating the terminal pathway via C5 cleavage into C5a and C5b. C3a and C5a are anaphylactic and chemotactic fragments involved in the recruitment of immune cells, while C3b and its degradation products are opsonins that facilitate phagocytosis. The C5b fragment binds with C6, C7, C8 and multiple units of C9 in the membrane, forming C5b-9, also known as the membrane attack complex (MAC), in the cell membranes of cells and microorganisms to cause cell lysis. Thus, complement activation ultimately results in three key outcomes: formation of the Membrane Attack Complex (MAC)

that promotes cell lysis and pro-inflammatory signal pathways; generation of C3a and C5a chemotactic peptides that activate and attract phagocytes; and stimulation of B cells, T cells, and follicular dendritic cells.

[0004] Persistent uncontrolled complement activation plays a major role in the pathogenesis of a subset of inflammatory and autoimmune diseases. As a result, systemic complement blockade has garnered significant attention as a therapeutic strategy for diseases including paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), cold agglutinin disease (CAD), C3 glomerulopathy (C3G), IgA nephropathy (IgAN), bullous pemphigoid (BP), geographic atrophy (GA), IgG4-related disease (IgG4 RD), or ANCA-associated vasculitis (AAV).

[0005] However, systemic inhibitors face two central challenges. First, because of complement's essential role in innate immunity, systemic inhibition increases patients' susceptibility to bacterial infections, including life-threatening meningococcal (*Neisseria meningitidis*) infection and sepsis, even in vaccinated patients. Second, circulating components of complement exist in high abundance and undergo rapid turnover, requiring high concentrations of systemic inhibitor to overcome this endogenous pharmacologic sink.

[0006] Together, these limitations necessitate a difficult balance between drug exposures high enough to effectively inhibit complement but low enough to minimize patients' infection risk, and substantial unmet need remains for safer and more effective anti-complement therapies.

[0007] Therefore, there is a need to develop reliable and effective treatment for complement-mediated diseases, ideally without compromising patients' ability to fight infection by avoiding systemic complement inhibition.

SUMMARY OF THE INVENTION

[0008] Provided is a method of treating a patient in need of reducing glomerular complement activity / activation, the method comprising administering a complement inhibitor (*e.g.*, a targeted complement inhibitor specific for a local tissue where there is complement activation) to the patient upon confirming that the patient has an elevated normalized urinary C5b-9 (uC5b-9) level compared to a control / reference standard.

[0009] Also provided is a method of identifying a patient as a candidate for treatment to reduce glomerular complement activity / activation, the method comprising determining a normalized urinary C5b-9 (uC5b-9) level in a urine sample from the patient, wherein an elevated level of the normalized urinary C5b-9 level, as compared to a control / reference

standard, identifies the patient as the candidate in need of treatment to reduce glomerular complement activity / activation.

[0010] Further provided is a method of adjusting treatment of a patient in need of reducing glomerular complement activity / activation, wherein the treatment comprises administering a complement inhibitor to the patient, the method comprising: adjusting a dose and/or a frequency of administration of the complement inhibitor, based on the extent the patient has an elevated normalized urinary C5b-9 (uC5b-9) level compared to a control / reference standard.

[0011] It should be understood that any one embodiment of the invention described herein, including those described only in the examples or claims, can be combined with any one or more additional embodiments of the invention, unless expressly disclaimed or is improper.

BRIEF DESCRIPTION OF THE DRAWINGS:

[0012] FIG. 1 is a schematic (not to scale) of the design of an exemplary targeted complement inhibitor used in the examples (C3d-mAb-2fH).

[0013] FIG. 2A shows the study design in the Passive Heymann Nephritis (PHN) model of kidney injury.

[0014] FIG. 2B shows time course of C3d deposition in Passive Heymann Nephritis (PHN) model by immunofluorescent staining. Kidney samples collected from the PHN model were immunostained for C3d deposition. Immunofluorescence from anti-C3d stained PHN kidneys shows clear C3d deposition in glomeruli by day 3 after anti-Fx1A-mediated disease induction. Immunofluorescence from at least 10 glomeruli from 3-4 rats per time point was measured by digital image analysis, and the results are presented herein as quantitation of C3d deposition in PHN glomeruli.

[0015] FIG. 2C shows that urine protein:creatinine ratio (uPCR) was elevated by day 3 in rats treated with anti-Fx1A nephrotoxic serum alone and continued to increase until end of study. Treatment with cobra venom factor (CVF), dosed daily beginning two days prior to anti-Fx1A instillation, significantly inhibited uPCR ($P < 0.0001$). Single IV doses of 1 to 30 mg/kg human C3d-mAb-2fH (ADX-097) inhibited uPCR progression ($P < 0.0001$). No dose-response was observed, suggesting that 1 mg/kg ADX-097 achieves maximal efficacy in this time frame. A 17 mg/kg dose (equimolar to 30 mg/kg ADX-097) of a non-targeted inhibitor, Fc-2fH₁₋₅, also inhibited uPCR progression.

[0016] FIG. 2D shows glomerular C3 fragment deposition quantification from anti-C3

fragment immunofluorescence on kidney tissue. Compared to non-disease controls, C3 fragment deposition was elevated in PHN rats treated with PBS ($P < 0.0001$). CVF treatment reduced anti-C3 fragment to levels equivalent to non-disease controls ($P < 0.0001$). ADX-097 treatment reduced glomerular C3 fragment deposition in a dose-dependent manner: 1 mg/kg ADX-097 reduced anti-C3 fragment immunofluorescence by approximately 40% ($P < 0.002$), and 3 mg/kg reduced by approximately 70% ($P < 0.0001$). ADX-097 doses ≥ 10 mg/kg, as well as 17 mg/kg Fc-2fH₁₋₅, reduced anti-C3 fragment immunofluorescence to levels equivalent to non-diseased controls.

[0017] FIG. 2E shows tissue and circulation drug exposure in Passive Heymann Nephritis. Kidney and plasma samples from the Passive Heymann Nephritis (PHN) study outlined in FIG. 2A were collected on study day 5 (48 hours after ADX-097 treatment), and were analyzed for presence of ADX-097. Plasma drug concentration was measured by drug-specific ELISA. Dose-dependent plasma drug concentrations were detected in the ADX-097 treatment groups. Fc-2fH₁₋₅ circulating concentrations were similar to the 30 mg/kg ADX-097 dose group, consistent with delivery of equimolar doses of the two proteins. A summary of circulating drug concentrations, expressed in nM and $\mu\text{g/ml}$, are listed in the table.

[0018] FIG. 2F shows that, compared to healthy controls, serum complement activity was slightly but not statistically significantly reduced in PHN rats treated with PBS. Activity was significantly inhibited in PHN rats treated with CVF, 50 mg/kg ADX-097, or 17 mg/kg Fc-2fH₁₋₅ (all $P < 0.0001$ vs. PHN + PBS). In contrast, compared to PHN + PBS, ADX-097 doses ≤ 10 mg/kg did not affect circulating complement.

[0019] FIG. 2G shows quantitation of glomerular immunofluorescence using an anti-fH antibody to detect localization of ADX-097. No anti-fH immunofluorescence is detected in non-disease controls, nor in PHN + PBS, PHN + CVF, or PHN + Fc-2fH₁₋₅ dose groups. Dose-dependent localization of ADX-097 is detected in glomeruli, with less ADX-097 localization detected in glomeruli from the 1 mg/kg IV dose group. No difference in glomerular drug localization is observed between the 3, 10, and 30 mg/kg ADX-097 dose groups.

[0020] FIGs. 3A-3D show correlation of urine C5b-9 with glomerular complement in Passive Heymann Nephritis. Soluble C5b-9 was measured in urine samples from study day 5 (48 hours after ADX-097 treatment) of the Passive Heymann Nephritis (PHN) study outlined in FIG. 2A. FIG. 3A shows that urine C5b-9/Creatinine ratio is dose-dependently reduced in PHN rats after treatment with ADX-097 (** $P < 0.002$, * $P < 0.03$). Note that these doses (1 to 10 mg/kg, IV) do not inhibit circulating complement (see FIG. 2F), suggesting that uC5b-

9/Cre reflects changes in renal complement activity. FIG. 3B shows X-Y correlation plot of glomerular C3 fragment immunostaining on study day 5 vs. urine C5b-9/Cre ratio. A strong correlation between glomerular complement deposition and urine C5b-9/Cre ratio ($P < 0.00000001$) was demonstrated. Similar to FIG. 3A, FIG. 3C shows that urine C5b-9/uPCR ratio is dose-dependently reduced in PHN rats after treatment with ADX-097. Similar to FIG. 3B, FIG. 3D shows X-Y correlation plot of glomerular C3 fragment immunostaining on study day 5 vs. urine C5b-9/uPCR ratio.

[0021] FIG. 4A is a summary of study design in the Passive Heymann Nephritis (PHN) model of kidney injury. PHN rats were treated with CVF starting on study day -2 (prior to disease induction by anti-Fx1A) or after onset of proteinuria on study day 3 with human C3d-mAb-2fH (ADX-097) at doses of 0.3, 1, or 3 mg/kg SC or with 1 mg/kg IV. Fc-2fH₁₋₅ was included in the study at SC doses equimolar to ADX-097.

[0022] FIG. 4B shows that ADX-097 treatment at doses ≥ 1 mg/kg showed similar reduction in proteinuria compared to CVF. At this time point, the 0.3 mg/kg group showed reduced uPCR, though less substantial than CVF or higher doses of ADX-097. At the end of study (day 7), all ADX-097 treated groups exhibited a dose-dependent reduction in proteinuria (* $P < 0.01$, ** $P < 0.005$, *** $P < 0.0001$ vs. anti-Fx1A + PBS).

[0023] FIG. 4C is a summary of uPCRAUC for days 3-7 of the study.

[0024] FIG. 4D shows time course of urine C5b-9/Cre ratio (uC5b-9), suggesting that 1 and 3 mg/kg doses of ADX-097 equivalently inhibit glomerular complement in the first 48 hours after dosing, but that effects on uC5b-9 are more durable in the 3 mg/kg dose group. AUC(uC5b-9) of 1 and 3 mg/kg ADX-097 is reduced compared to the untreated anti-Fx1A dose group ($P < 0.03$ for 1 mg/kg SC and IV; $P < 0.002$ for 3 mg/kg SC). AUC(uC5b-9) of the 0.3 mg/kg ADX-097 dose group and of the 0.17 and 0.57 mg/kg Fc-2fH dose groups are not statistically significantly different from that of untreated anti-Fx1A, indicating little effect of these treatments on uC5b-9. AUC(uC5b-9) of the 1.7 mg/kg Fc-2fH dose group is reduced relative to untreated anti-Fx1A ($P < 0.008$), likely reflecting the longer circulating half-life of Fc-2fH.

[0025] FIG. 4E shows comparison of 3 mg/kg ADX-097 to the molar equivalent dose of Fc-2fH₁₋₅ (1.7 mg/kg). Both molecules reduce uPCR equivalently up to day 5, but by day 7 the effect of ADX-097 is more potent than that of Fc-2fH₁₋₅ (+ $P < 0.05$).

[0026] FIGs. 5A-5D show correlation of urine C5b-9 with glomerular complement in Passive Heymann Nephritis study described in FIG. 4A. Soluble C5b-9 was measured in urine samples from study day 7 (96 hours after ADX-097 treatment) of the Passive Heymann

Nephritis (PHN) study outlined in FIG. 4A. FIG. 5A shows that urine C5b-9/Creatinine ratio is dose-dependently reduced in PHN rats after treatment with ADX-097. FIG. 5B shows X-Y correlation plot of glomerular C3 fragment immunostaining on study day 7 vs. urine C5b-9/Cre ratio. A strong correlation between glomerular complement deposition and urine C5b-9/Cre ratio was demonstrated. Similar to FIG. 5A, FIG. 5C shows that urine C5b-9/uPCR ratio is dose-dependently reduced in PHN rats after treatment with ADX-097. Similar to FIG. 5B, FIG. 5D shows X-Y correlation plot of glomerular C3 fragment immunostaining on study day 7 vs. urine C5b-9/uPCR ratio.

[0027] FIGs. 6A and 6B show Spearman correlation matrix of the various urinary biomarkers to glomerular C3, based on data obtained from the PHN Study depicted in FIG. 2A (FIG. 6A) and the PHN Study depicted in FIG. 4A (FIG. 6B).

[0028] FIGs. 7A-7F show that ADX-097 protects podocyte ultrastructure in Passive Heymann Nephritis (PHN). (FIG. 7A) Representative EM image from glomeruli in the healthy control (treated with normal serum) group shows well-differentiated podocyte foot processes (white arrows). (FIG. 7B) Enlarged image of the area outlined in the white frame in FIG. 7A. White arrows indicate examples of normal slit diaphragms. The glomerular basement membrane (GBM) is of uniform thickness with a distinct lamina densa. (FIG. 7C) Representative glomerular EM image from a PHN rat shows extensive foot process effacement (yellow arrows), electron-dense regions consistent with immune complexes (yellow asterisks). (FIG. 7D) Enlarged image of the area outlined in the white box in FIG. 7B. Yellow arrows indicate effaced podocyte foot processes. Yellow asterisks denote electron-dense regions consistent with immune complex deposition. Note the distorted and thickened GBM without a clear lamina densa. (FIG. 7E) Glomerular EM from PHN rats treated with 3 mg/kg SC ADX-097 show substantial preservation of podocyte foot processes (white arrows), though occasional examples of effaced podocytes can be found (yellow arrows). (FIG. 7F) Enlarged image of the area within the white box in FIG. 7C. The white arrow highlights a representative healthy slit diaphragm, while the yellow arrow points out a partially effaced podocyte foot process. The GBM is more uniform in thickness and has a differentiated lamina densa.

[0029] FIGs. 8A-8C show the results of evaluating the effects of ADX-097 on podocyte ultrastructure using stimulated emission depletion (STED) super resolution microscopy of Nephritin-immunostained kidney sections. The Nephritin protein is expressed in podocytes and localizes to the slit diaphragm, and immunostaining therefore delineates podocyte foot processes in Nephritin-immunostained sections. FIG. 8A shows a representative image from a

healthy glomerulus, where Nephrit immunofluorescence outlines the interdigitating podocyte foot processes (white arrows). In kidneys collected from PHN rats, Nephrit-immunostaining reveals the extent of foot process effacement (FIG. 8B), with a dysregulated slit diaphragm surrounding large unstained areas that are consistent with heavily effaced podocytes (white arrows, FIG. 8B). In PHN rats that have been treated with 3 mg/kg ADX-097, the structure of the Nephrit-stained slit diaphragm is largely restored, indicating that ADX-097 preserves podocyte ultrastructure in this model (FIG. 8C).

DETAILED DESCRIPTION

[0030] While there has been investigation as to whether urine soluble C5b-9 (uC5b-9) is suitable as a marker for complement activation in patients, large individual variabilities have been observed, thus complicating data analysis and interpretation. Perhaps more importantly, investigation has focused on serum or plasma (*e.g.*, systemic) complement activation with respect to uC5b-9 levels. No correlation has been established between uC5b-9 level and local tissue (such as kidney) complement activation, either alone or normalized against proteinuria or urine creatinine (uCr) level. Thus, important questions remained as to how independent uC5b-9 is from proteinuria, and whether changes in uC5b-9 reflect systemic or local tissue (such as glomerular) complement activation.

[0031] It has been found that targeted delivery of a complement inhibitor could *locally* concentrate the drug where complement is active, thus avoiding *systemic* inhibition by minimizing the circulating drug exposure required for efficacy. Moreover, data presented herein demonstrates that urinary C5b-9 (uC5b-9) is a highly accurate biomarker that is well correlated with complement activity in local tissues affected by complement-mediated diseases (*e.g.*, those with a kidney damage component). Thus uC5b-9 can be used as a surrogate marker to monitor local tissue disease activity, and guide treatment decisions, such as treatment using complement inhibitors targeted to such local tissues affected by the diseases, without systemic complement inhibition.

[0032] Specifically, data presented herein demonstrates that there is a strong correlation between reduction in uC5b-9 level and local tissue (*e.g.*, glomerular)-specific complement inhibition (such as evidenced by local *e.g.*, glomerular, C3 immunostaining), in that dose-responsive complement inhibition observed in local tissue is associated with a dose-responsive urine C5b-9 reduction, indicating that uC5b-9 is a urinary marker of glomerular complement. Further, uC5b-9 level normalized to either uCr or uPCR (urinary proteinuria creatinine ratio) most directly reflects dose-dependent treatment response in glomeruli,

suggesting that uC5b-9 normalized against uCr or uPCR can be used as a fluid biomarker of glomerular complement activity that may be useful for, *e.g.*, clinical trial and/or treatment guidance / monitoring, including dose finding in clinical trials, and/or dose adjustment and treatment efficacy monitoring during treatment.

[0033] An exemplary targeted complement inhibitor is a conjugate comprising a complement inhibitor - Factor H – and an antibody specific for C3d deposited in local tissues, such as kidney. One such targeted complement inhibitor, as exemplified herein in the examples, is ADX-097, a bi-functional fusion protein comprising a humanized anti-C3d monoclonal antibody linked to two moieties of the first five consensus repeats of factor H (fH₁₋₅). ADX-097 was designed to locally inhibit complement activation in diseased tissue while minimizing systemic blockade.

[0034] Thus, provided is the use of urinary C5b-9 (uC5b-9) as a biomarker for effective treatment of complement-mediated diseases (*e.g.*, those with a kidney damage component) using complement inhibitors targeted to local tissues affected by the diseases, without systemic complement inhibition.

[0035] The data presented herein demonstrates that urinary C5b-9 (uC5b-9) correlates strongly with glomerular complement activity, and is independent of urinary protein-creatinine ratio (uPCR), suggesting the utility of uC5b-9 as a biomarker for local complement activity.

[0036] The uPCR test is a urine test that measures the levels of protein and creatinine in a urine sample. Creatinine is a waste product produced by muscle cells as they use creatinine (a natural chemical that gives muscles energy). When kidneys function normally, waste products including creatinine are filtered out of blood, and exit body with urine. Large molecular weight proteins, however, are not normally filtered out to the urine through kidney (that is, kidneys filter some proteins but also return proteins back to the blood). Thus, the normal uPCR is very low. However, a damaged or improperly functioning kidney may not be able to retain certain proteins in the blood, and instead filter the protein into urine, thus increasing the uPCR value. Therefore, the uPCR test provides a healthcare provider important information about how kidneys function. The results of this test help healthcare providers diagnose conditions that can cause kidney damage. uPCR is also used to monitor the efficacy of certain treatments.

[0037] uPCR is a measure of kidney function that strongly correlates with damaged kidney's filtration ability that permits passing through of large molecule proteins into urine (which does not occur in normal kidney), while such damaged kidney filtration ability is not

fully restored even after local tissue complement activity has subsided due to, for example, effective treatment by targeted complement inhibitors.

[0038] Meanwhile, uC5b-9 is a measure of complement activity in local tissues such as the kidney. Compared to uPCR, uC5b-9 measures a different aspect of kidney injury.

[0039] Thus, provided is a method of treating a patient in need of reducing glomerular complement activity / activation, the method comprising administering a complement inhibitor (*e.g.*, a targeted complement inhibitor specific for a local tissue where there is complement activation) to the patient upon confirming that the patient has an elevated normalized urinary C5b-9 (uC5b-9) level compared to a control / reference standard.

[0040] Also provided is a method of adjusting treatment of a patient in need of reducing glomerular complement activity / activation, wherein the treatment comprises administering a complement inhibitor to the patient, the method comprising: adjusting a dose and/or a frequency of administration of the complement inhibitor, based on the extent the patient has an elevated normalized urinary C5b-9 (uC5b-9) level compared to a control / reference standard.

[0041] In some embodiments, the normalized urinary C5b-9 (uC5b-9) level in the patient is normalized against uCr (urinary Creatinine) and/or uPCR (urinary Protein to Creatinine Ratio).

[0042] In some embodiments, the patient has a complement-mediated disease having complement activity / activation in kidney.

[0043] In some embodiments, the complement-mediated disease is focal segmental glomerulosclerosis, glomerulonephritis, complement 3 glomerulopathy (C3G), membranoproliferative glomerulonephritis, C3 glomerulonephritis, type II membranoproliferative glomerulonephritis (MPGN II), membranous nephropathy (MN), IgA nephropathy (IgAN), hypertensive nephropathy, diabetic nephropathy, thrombotic microangiopathy, lupus nephritis (LN), hemolytic uremic syndrome (HUS), atypical hemolytic uremic syndrome (aHUS), diabetic retinopathy, IgG4-related disease (IgG4 RD), or ANCA-associated vasculitis (AAV).

[0044] In some embodiments, the complement-mediated disease is AAV, IgAN, lupus nephritis (LN), or C3G.

[0045] In some embodiments, the complement inhibitor is a tissue-specific complement inhibitor (*e.g.*, not a systemic complement inhibitor).

- [0046] In some embodiments, the complement inhibitor specifically targets kidney.
- [0047] In some embodiments, the complement inhibitor comprises a binding moiety specific for C3 deposit in a disease tissue.
- [0048] In some embodiments, the binding moiety is an antibody or an antigen-binding portion thereof specific for C3d.
- [0049] In some embodiments, the complement inhibitor comprises factor H, CR1, DAF, MCP, Crry, MAp44, MAp19, CD59, or a biologically active fragment thereof.
- [0050] In some embodiments, the complement inhibitor comprises: (i) two heavy chain-containing polypeptides, each comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 282, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 72 or 41; and, (ii) two light chain-containing polypeptides, each comprising the amino acid sequence of SEQ ID NO: 279.
- [0051] In some embodiments, the method further comprises ceasing or discontinuing treatment (*e.g.*, ceasing or discontinuing administering the complement inhibitor to the patient) upon confirming that the patient has normal level of normalized urinary C5b-9 (uC5b-9) compared to the control / reference standard.
- [0052] In some embodiments, the patient has elevated normalized uPCR at the time of stopping / discontinuing treatment.
- [0053] Also provided is a method of identifying a patient as a candidate for treatment to reduce glomerular complement activity / activation, the method comprising determining a normalized urinary C5b-9 (uC5b-9) level in a urine sample from the patient, wherein an elevated level of the normalized urinary C5b-9 level, as compared to a control / reference standard, identifies the patient as the candidate in need of treatment to reduce glomerular complement activity / activation.
- [0054] In some embodiments, the normalized urinary C5b-9 (uC5b-9) level in the patient is normalized against uCr (urinary Creatinine) and/or uPCR (urinary Protein to Creatinine Ratio).
- [0055] In some embodiments, the patient has, or is at risk of having, a complement-mediated disease having complement activity / activation in kidney.
- [0056] In some embodiments, the complement-mediated disease is focal segmental glomerulosclerosis, glomerulonephritis, complement 3 glomerulopathy (C3G), membranoproliferative glomerulonephritis, C3 glomerulonephritis, type II membranoproliferative glomerulonephritis (MPGN II), membranous nephropathy (MN), IgA nephropathy (IgAN), hypertensive nephropathy, diabetic nephropathy, thrombotic

microangiopathy, lupus nephritis (LN), hemolytic uremic syndrome (HUS), atypical hemolytic uremic syndrome (aHUS), diabetic retinopathy, IgG4-related disease (IgG4 RD), or ANCA-associated vasculitis (AAV).

[0057] In some embodiments, the complement-mediated disease is AAV, IgAN, lupus nephritis (LN), or C3G.

[0058] The methods described herein comprise administering a complement inhibitor to the patient upon confirming that the patient has an elevated normalized urinary C5b-9 (uC5b-9) level compared to a control / reference standard. In some embodiments, the complement inhibitor is a targeted complement inhibitor or tissue-specific complement inhibitor, specific for a local tissue where there is complement activation. In some embodiments, the complement inhibitor inhibits complement activation or activity in local tissue (such as in kidney), without substantially inhibit systemic complement activation or activity.

[0059] Exemplary complement inhibitors include, without limitation, the fusion protein constructs for complement associated diseases as described in WO2020123662, the entire content of which is incorporated herein by reference.

[0060] For example, in some embodiments, the subject complement inhibitor is targeted to kidney where there is C3 deposit, such as kidney, liver, or skin.

[0061] In some embodiments, the complement inhibitor comprises a targeting domain or targeting moiety that specifically binds to a moiety or marker at a local tissue (*e.g.*, such as kidney, liver, or skin) where there is complement activity.

[0062] In some embodiments, the targeting domain is an antibody or an antigen-binding fragment thereof specific for the moiety or marker.

[0063] In some embodiments, the complement inhibitor comprises a complement modulator, such as factor H, CR1, DAF, MCP, Crry, MAp44, MAp19, CD59, or a biologically active fragment thereof.

[0064] In some embodiments, the targeting domain, such as the antibody or antigen-binding fragment thereof, is linked to the complement modulator (*e.g.*, factor H, CR1, DAF, MCP, Crry, MAp44, MAp19, CD59, or a biologically active fragment thereof) via a linker, such as a flexible peptide linker.

[0065] In some embodiments, the complement modulator may be selected from human MCP, human DAF, mouse DAF, human CD59, mouse CD59 isoform A, mouse CD59 isoform B, mouse Crry protein, human CR1, human factor H, mouse factor H, or a biologically active fragments thereof, and variants thereof.

[0066] As used herein, the term “complement receptor 1,” “CR1,” or “CD35” can refer to

a human gene encoding a protein of 2039 amino acids, with a predicted molecular weight of 220 kilodaltons ("kDa"), including homologs thereof. The gene can be expressed principally on erythrocytes, monocytes, neutrophils, and B cells, but may also be present on some T lymphocytes, mast cells, and glomerular podocytes. CR1 protein can be typically expressed at between 100 and 1000 copies per cell. CR1 can be the main system for processing and clearance of complement-opsonized immune complexes. CR1 can negatively regulate the complement cascade, mediate immune adherence and phagocytosis, and inhibit all complement pathways. The full-length CR1 protein may comprise a 42 amino acid signal peptide, an extracellular domain of 1930 amino acids, a 25 amino acid transmembrane domain, and a 43 amino acid C-terminal cytoplasmic domain. The extracellular domain of CR1 can include 25 potential N-glycosylation signal sequences and may comprise 30 short consensus repeat ("SCR") domains, also known as complement control protein (CCP) repeats, or sushi domains, each 60 to 70 amino acids long. The sequence homology between SCRs can range between 60-99 percent. The 30 SCR domains may further be grouped into four longer regions termed long homologous repeats ("LHRs"), each encoding approximately 45 kDa segments of the CR1 protein, designated LHR-A, -B, -C, and -D (see, *e.g.*, Krych-Goldberg *et al.*, 274(44): 31160-31168, 1999). The first three LHRs can comprise seven SCR domains each, while LHR-D can comprise 9 SCR domains. The active sites on the extracellular domain of CR1 protein can include a C4b-binding site with lower affinity for C3b in SCR 1-3 comprising amino acids 42-234, a C3b-binding site with lower affinity for C4b in SCRs 8-11 comprising amino acids 490-745, a C3b-binding site with lower affinity for C4b in SCRs 15-18 comprising amino acids 940-1196, and a C1q-binding site in SCRs 22-28 comprising amino acids 1394-1842.

[0067] SEQ ID NO: 8 represents an exemplary sequence for the full-length human CR1 (see, *e.g.*, UniProtKB/Swiss-Prot. Accession No. P17927). Amino acids 1-41 may correspond to the signal peptide, amino acids 42-2039 may correspond to the mature protein, comprising amino acids 42-1971, that may correspond to the extracellular domain, amino acids 1972-1996, that may correspond to the transmembrane domain, and amino acids 1997-2039, that may correspond to the cytoplasmic domain. In the extracellular domain, amino acids 42-101 may correspond to SCR 1, 102-163 may correspond to SCR 2, amino acids 164-234 may correspond to SCR 3, amino acids 236-295 may correspond to SCR 4, amino acids 295-355 may correspond to SCR 5, amino acids 356-418 may correspond to SCR 6, amino acids 419-489 may correspond to SCR 7, amino acids 491-551 may correspond to SCR 8, amino acids 552-613 may correspond to SCR 9, amino acids 614-684 may correspond to

SCR 10, amino acids 686-745 may correspond to SCR 11, amino acids 745-805 may correspond to SCR 12, amino acids 806-868 may correspond to SCR 13, amino acids 869-939 may correspond to SCR 14, amino acids 941-1001 may correspond to SCR 15, amino acids 1002-1063 may correspond to SCR 16, amino acids 1064-1134 may correspond to SCR 17, amino acids 1136-1195 may correspond to SCR 18, amino acids 1195-1255 may correspond to SCR 19, amino acids 1256-1318 may correspond to SCR 20, amino acids 1319-1389 may correspond to SCR 21, amino acids 1394-1454 may correspond to SCR 22, amino acids 1455-1516 may correspond to SCR 23, amino acids 1517-1587 may correspond to SCR 24, amino acids 1589-1648 may correspond to SCR 25, amino acids 1648-1708 may correspond to SCR 26, amino acids 1709-1771 may correspond to SCR 27, amino acids 1772-1842 may correspond to SCR 28, amino acids 1846-1906 may correspond to SCR 29, amino acids 1907-1967 may correspond to SCR 30. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that CR1 protein or biologically active fragments thereof can encompass all species and strain variations.

[0068] As used herein, the term “biologically active” fragment of CR1 protein can refer to any soluble fragment of CR1 lacking the transmembrane domain and the cytoplasmic domain, including fragments comprising, consisting essentially of or consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 SCR domains, including any fragments of the full-length CR1 protein having some or all the complement inhibitory activity of the full-length CR1 protein. Functional fragments may include SCRs 1 and 2; SCRs 1, 2, 3, and 4; SCRs 1, 2, 3, 4, 5, 6, 7; SCRs 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 (“CR1 (1-10)”); SCRs 6, 7, 8, 9, 10, 11, and 12; SCRs 8 and 9; SCRs 8, 9, 10, and 11; SCRs 8, 9, 10, 11, 12, 13, and 14; SCRs 15 and 16; SCRs 12, 13, 14, 15, 16, and 17; SCRs 15, 16, 17, 18, and 19; SCRs 1 through 17 (“CR1 (1-17)”); SCRs 1 through 23; SCRs 1 through 28. Example variant polypeptides comprise at least three SCRs of each of domains A and B; at least three SCRs of each of domains A, B and C; at least the first three SCRs of domains A, B, and C, or amino acid sequences at least 90% identical to any of the foregoing.

[0069] In some embodiments, the complement modulator peptide comprises complement receptor 1 (CR1) protein. In some embodiments, the complement modulator peptide comprises domain A of the CR1 protein or a fragment thereof that retains at least three short consensus repeats (SCRs) of domain A. In some embodiments, the complement modulator peptide comprises domain B of the CR1 protein or a fragment thereof that retains at least three SCRs of domain B. In some embodiments, the complement modulator peptide comprises domain C of the CR1 protein or a fragment thereof that retains at least three SCRs

of domain C. In some embodiments, the pharmaceutical composition further comprises domain D of the CR1 protein or a fragment thereof that retains at least three SCRs of domain D. In some embodiments, the complement modulator peptide comprises first three SCRs of domain A, first three SCRs of domain B, and first three SCRs of domain C of the CR1 protein. In some embodiments, the CR1 protein is a human CR1 protein. In some embodiments, the complement modulator polypeptide is CR1 (1-10). In some embodiments, the complement modulator polypeptide is CR1 (1-17). In some embodiments, the CR1 (1-10) comprises the amino acid sequence of SEQ ID NO: 41 or SEQ ID NO: 91, or a variant thereof with an amino acid sequence at least 85% identical. In some embodiments, the CR1 (1-17) comprises the amino acid sequence of SEQ ID NO: 42 or SEQ ID NO: 92, or a variant thereof with an amino acid sequence at least 85% identical.

[0070] In some embodiments, the complement modulator peptide is a decay-accelerating factor (DAF) or a biologically active fragment thereof. In some embodiments, the DAF is a human DAF. In some embodiments, the biologically active fragment of human DAF comprises at least one of a short consensus repeat (SCR) domain and an O-glycosylated serine/threonine-rich domain of a full-length human DAF. In some embodiments, the biologically active fragment of human DAF comprises SCR 1 to 4, or SCR 2 to 4 of a full-length human DAF. In some embodiments, the biologically active fragment of human DAF comprises the amino acid sequence of SEQ ID NO: 184, or a variant thereof with an amino acid sequence at least 85% identical.

[0071] As used herein, the terms “complement factor H,” “factor H,” or “FH” can refer to complement factor H, a single polypeptide chain plasma glycoprotein, including homologs thereof. The protein may be composed of 20 conserved short consensus repeat (SCR) domains of approximately 60 amino acids, arranged in a continuous fashion like a string of beads, separated by short linker sequences of 2-6 amino acids each. Factor H may bind to C3b, accelerate the decay of the alternative pathway C3-convertase (C3bBb) as well as the alternative pathway C5 convertase (C3bBb3b), and act as a cofactor for the proteolytic inactivation of C3b. In the presence of factor H, proteolysis by factor I may result in the cleavage and inactivation of C3b. Factor H can have at least three distinct binding domains for C3b, which may be located within any one of SCRs 1-20, SCRs 1-4, SCRs 5-8, and SCRs 19-20. Each domain may bind to a distinct region within the C3b protein: the N-terminal sites may bind to native C3b; the second site, located in the middle region of factor H, may bind to the C3c fragment and the site located within SCR19 and 20 may bind to the C3d region. In addition, factor H can also contain binding sites for heparin, which may be located

within SCR 7, SCRs 5-12, and SCR 20 of factor H and may overlap with those of the C3b binding sites. Structural and functional analyses have shown that the domains for the complement inhibitory activity of factor H may be located within the first four N-terminal SCR domains.

[0072] SEQ ID NO: 9 represents an exemplary amino acid sequence for the full-length human factor H protein (see, *e.g.*, UniProtKB/Swiss-Prot. Accession No. P08603); SEQ ID NO: 10 represents an exemplary amino acid sequence for the full-length mouse factor H protein (see, *e.g.*, UniProtKB/Swiss-Prot. Accession No. P06909). In the human factor H sequence, amino acids 1-18 of SEQ ID NO: 9 may correspond to the signal peptide, and amino acids 19-1231 of SEQ ID NO: 9 may correspond to the mature protein. Within that protein, amino acids 21-80 of SEQ ID NO: 9 may correspond to SCR 1, amino acids 85-141 of SEQ ID NO: 9 may correspond to SCR 2, amino acids 146-205 of SEQ ID NO: 9 may correspond to SCR 3, amino acids 210-262 of SEQ ID NO: 9 may correspond to SCR 4, and amino acids 267-320 of SEQ ID NO: 9 may correspond to SCR 5. In the mouse factor H sequence, amino acids 1-18 of SEQ ID NO: 10 may correspond to the signal peptide, and amino acids 19-1234 of SEQ ID NO: 10 may correspond to the mature protein. Within that protein, amino acids 19-82 of SEQ ID NO: 10 may correspond to SCR 1, amino acids 83-143 of SEQ ID NO: 10 may correspond to SCR 2, amino acids 144-207 of SEQ ID NO: 10 may correspond to SCR 3, amino acids 208-264 of SEQ ID NO: 10 may correspond to SCR 4, and amino acids 265-322 of SEQ ID NO: 10 may correspond to SCR 5. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that factor H or biologically active fragments thereof can encompass all species and strain variations.

[0073] As used herein, the term “biologically active” fragment of factor H can refer to any portion of a factor H protein having some or all the complement inhibitory activity of the full-length factor H protein, and can include, but is not limited to, factor H fragments comprising SCRs 1-4, SCRs 1-5, SCRs 1-8, SCRs 1-18, SCRs 19-20, or any homolog of a naturally-occurring factor H or fragment thereof, as described in detail below. In some examples of the fusion protein constructs, the biologically active fragment of factor H may have one or more of the following properties: (1) binding to C-reactive protein (CRP), (2) binding to C3b and/or its fragments, (3) binding to heparin, (4) binding to sialic acid, (5) binding to endothelial cell surfaces, (6) binding to cellular integrin receptor, (7) binding to pathogens, (8) C3b co-factor activity, (9) C3 and C5 alternative pathway convertase decay-acceleration activity, and (10) inhibiting the alternative complement pathway.

[0074] In some embodiments, the complement modulator peptide is factor H or a biologically active fragment thereof. In some embodiments, the factor H is a human factor H. In some embodiments, the biologically active fragment of human factor H comprises a stretch of amino acids selected from the group consisting of: amino acids 21-266, amino acids 21-320, amino acids 21-509 or amino acids 19-1106 of SEQ ID NO: 9, or a variant thereof with an amino acid sequence at least 85% identical to the stretch of amino acids. In some embodiments, the biologically active fragment of human factor H comprises one or more groups of short consensus repeats (SCRs) comprising SCRs 1 to 20, SCRs 1 to 2, SCRs 2 to 3, SCRs 3 to 4, SCRs 4 to 5, SCRs 5 to 6, SCRs 6 to 7, SCRs 7 to 8, SCRs 8 to 9, SCRs 9 to 10, SCRs 10 to 11, SCRs 11 to 12, SCRs 12 to 13, SCRs 13 to 14, SCRs 14 to 15, SCRs 15 to 16, SCRs 16 to 17, SCRs 17 to 18, SCRs 19 to 20 of a full-length human factor H, or any combination of SCRs 1 to 20.

[0075] In some embodiments, the biologically active fragment of human factor H comprises SCRs 1 to 4 of a full-length human factor H. In some embodiments, the biologically active fragment of human factor H comprises SCRs 1 to 5 of a full-length human factor H. In some embodiments, the biologically active fragment of human factor H comprises the amino acid sequence of SEQ ID NO: 72 or SEQ ID NO: 108, or a variant thereof with an amino acid sequence at least 85% identical.

[0076] In some embodiments, the complement modulator peptide is MCP or a biologically active fragment thereof. In some embodiments, the MCP is a human MCP. In some embodiments, the biologically active fragment of human MCP comprises at least one of a short consensus repeat (SCR) domain of a full-length human MCP. In some embodiments, the biologically active fragment of human MCP comprises SCRs 3 to 4 of the full-length human MCP. In some embodiments, the biologically active fragment of human MCP comprises the amino acid sequence of SEQ ID NO: 187, or a variant thereof with an amino acid sequence at least 85% identical.

[0077] In some embodiments, the complement modulator peptide is Map44 or a biologically active fragment thereof. In some embodiments, the Map44 is a human Map44. In some embodiments, the Map44 comprises the amino acid sequence of SEQ ID NO: 186, or a variant thereof with an amino acid sequence at least 85% identical.

[0078] In some embodiments, the complement modulator peptide is Map19 or a biologically active fragment thereof. In some embodiments, the Map19 is a human Map44. In some embodiments, the Map44 comprises the amino acid sequence or a variant with at least 85% identical to human Map19.

[0079] In some embodiments, the complement modulator peptide is CD59 or a biologically active fragment thereof. In some embodiments, the CD59 is a human CD59. In some embodiments, the CD59 comprises the amino acid sequence of SEQ ID NO: 185, or a variant thereof with an amino acid sequence at least 85% identical.

[0080] In some embodiments, the complement modulator peptide is Crry or a biologically active fragment thereof. SEQ ID NO: 7 represents an exemplary sequence for the full-length mouse Crry protein. Amino acids 1-40 may correspond to the leader peptide, amino acids 41-483 of SEQ ID NO: 7 may correspond to the mature protein, comprising amino acids 41-405 of SEQ ID NO: 7, that may correspond to the extracellular domain, amino acids 406-426 of SEQ ID NO: 7, that may correspond to the transmembrane domain, and amino acids 427-483 of SEQ ID NO: 7, that may correspond to the cytoplasmic domain. In the extracellular domain, amino acids 83-143 of SEQ ID NO: 7 may correspond to SCR 1, amino acids 144-205 of SEQ ID NO: 7 may correspond to SCR 2, amino acids 206-276 of SEQ ID NO: 7 may correspond to SCR 3, amino acids 277-338 of SEQ ID NO: 7 may correspond to SCR 4, and amino acids 339-400 of SEQ ID NO: 7 may correspond to SCR 5. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that mouse Crry protein or biologically active fragments thereof can encompass all species and strain variations. As used herein, the term “biologically active” fragment of Crry protein can refer to any soluble fragment of Crry lacking the transmembrane domain and the cytoplasmic domain, including fragments comprising, consisting essentially of or consisting of 1, 2, 3, 4, or 5 SCR domains, including any fragments of the full-length Crry protein having some or all the complement inhibitory activity of the full-length Crry protein.

[0081] The targeting moiety of the complement inhibitor can be responsible for targeted delivery of the complement inhibitor to the sites of action, such as the local site of complement activation. There, the complement modulator can have a therapeutic activity such as specifically inhibiting complement activation at the local site, preferably without inhibiting systemic complement activation. The complement inhibitor constructs described herein thus generally has the dual functions of binding to a target by the targeting moiety, such as an epitope recognized by an antibody described herein, and exerting therapeutic activity through inhibition of complement activation.

[0082] In some embodiments, the targeting moiety can be an antibody or an antigen binding fragment thereof that is human, murine, humanized, or camelized.

[0083] In some embodiments, the epitope recognized by the antibody or antigen binding fragment thereof can be a domain of a mammalian annexin protein, a phospholipid, such as

one or more of the C2 antibody-reactive phospholipids described below, or a complement protein, such as C3d, C3 fragments (*e.g.*, deposited C3 fragments - C3b, iC3b, C3d, C3dg; free or undeposited C3 fragments- C3a, C3b, C3c or C3f).

[0084] In some embodiments, the antibody or antigen-binding fragment thereof can specifically bind to a domain of a mammalian annexin protein. The annexins are a family of calcium- (Ca^{2+}) and phospholipid-binding proteins that differ from most other Ca^{2+} binding proteins in their Ca^{2+} binding sites. The annexin family Ca^{2+} binding site has a unique architecture that enables annexin family members to reversibly dock onto the periphery of cellular and/or organellar membranes. The conserved Ca^{2+} binding site characteristic of annexin family members is located in the annexin core domain, and comprises four annexin repeats, each seventy (70) amino acids long. The annexin core domain is α -helical and forms a compact, curved disc with a convex surface comprising the Ca^{2+} and membrane-binding sites and a concave side oriented away from the membrane that is available for other types of interaction. Annexin family members also typically have an amino- terminal domain of variable length that precedes the annexin core domain and is diverse in sequence and structure. Twelve annexin subfamilies have been characterized in vertebrates, including annexin IV and annexin 2, each having different splice variants, with different amino-terminal domains and differently positioned Ca^{2+} binding sites. The antibody or antigen-binding fragment thereof, that specifically binds to a domain within or recognizes an epitope within the annexin IV protein, can be a B4 mAb or an antigen binding fragment derived from the B4 mAb. The antibody or antigen-binding fragment thereof, that specifically binds to a domain within or recognizes an epitope within the annexin IV protein (*e.g.*, human annexin IV protein), can be a B4 mAb or an antigen binding fragment derived from the B4 mAb, described in Kulik *et al.*, J Immunol. 182(9): 5363 (2009). Exemplary CDRs of B4 mAb are provided in SEQ ID NOS: 11-16. The targeting moiety can further be an antibody or an antigen binding fragment thereof that specifically binds to or recognizes an epitope within the annexin 2 protein (*e.g.*, human annexin 2 protein).

[0085] In some embodiments, the antibody or antigen-binding fragment thereof can also specifically bind to a phospholipid (*e.g.*, phosphatidylethanolamine (PE), cardiolipin (CL), phosphatidylcholine (PC), phosphatidylinositol, phosphatidylglycerol, phosphatidylserine, or phosphatidic acid) or malondialdehyde (MDA). The antibody or antigen-binding fragment thereof, that specifically binds to a phospholipid, can be a C2 mAb or derivative thereof. The phospholipid can be present on the surface of a cell, a basement membrane (*e.g.*, Bruch's membrane), or in a pathological structure (*e.g.*, drusen) in an individual that is in or adjacent

to a tissue undergoing (or at risk of undergoing) tissue injury (such as nonischemic injury), oxidative damage, or any combinations thereof. The phospholipid can be neutral, negatively or positively charged, or oxidized. The antibody or antigen-binding fragment thereof, that specifically binds to a phospholipid, can be a C2 mAb, or an antigen binding fragment derived from the C2 mAb, described in Elvington *et al.*, J Immunol., 188(3): 1460-1468 (2012). Exemplary CDRs of C2 mAb are provided in SEQ ID NOS: 17-22. C2 mAb recognizes a subset of phospholipids that are exposed after complement activation or ischemia; this subset of phospholipids is referred to herein as “C2 antibody-reactive phospholipids.” C2 mAb has been shown to recognize a subset of phospholipids that included phosphatidylcholine, phosphatidylethanolamine and cardiolipin, but not phosphatidylglycerol or phosphatidylserine.

[0086] In some embodiments, the targeting moiety, in some cases, can be an antibody or an antigen-binding fragment thereof, which specifically binds to deposited or opsonized C3 fragments, *e.g.*, C3b, iC3b, C3d or C3dg, but may or may not bind to free or circulating or undeposited C3 fragments, *e.g.*, C3a, C3b, C3c or C3f. In some examples, it is possible for fusion protein constructs comprising an anti-C3d or anti-C3dg antibody or an antigen-binding fragment thereof to bind with relatively higher affinity to deposited C3 fragments compared to free C3 or C3 fragments. For example, the antibody or antigen-binding fragment thereof can bind C3 and C3b with about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold, or about 10-fold lower binding affinity compared to C3d. In some embodiments, the antibody or antigen-binding fragment binds C3 and/or C3b with a K_D of 10^{-4} M or higher, 10^{-3} M, or higher, or 10^{-2} M or higher, and binds iC3b, C3dg, or both, with a binding affinity (K_D) of 10^{-8} M or less, 10^{-9} M or less, or 10^{-10} M or less.

[0087] In some embodiments, it is possible for the subject complement inhibitor constructs comprising an anti-C3d or anti-C3dg antibody or an antigen-binding fragment thereof to bind to deposited C3 fragments as well as free C3 or C3 fragments. It is further possible that the anti-C3d or anti-C3dg antibody or an antigen-binding fragment thereof, of an exemplary complement inhibitor construct, can bind to complement fragment C3d and have the ability to discriminate between tissue bound C3 fragments from circulating C3 (*e.g.*, C3, C3b, or C3 (H2O)). Examples of the anti-C3d or anti-C3dg antibodies or antigen binding fragments thereof include, but are not limited to, mAbs 3d9a, 3d29 and 3d8b. In some instances, the anti-C3d or anti-C3dg antibodies of this disclosure can bind to C3d with greater specificity than commercially available anti-C3d antibodies, such as, for example, anti-C3d antibodies designated by the Quidel catalog numbers A207 and A250, that are commercially

available from the Quidel Corporation (Quidel Corp., San Diego and Santa Clara, Calif.). In some cases, the targeting moiety can comprise an antibody specific to C3d and/or other C3 fragments (C3b, iC3b, C3c, C3dg, etc.), such as an antibody C8D3. The antibody C8D3 can bind to an epitope on C3d with high affinity, which overlaps with the CR2 binding epitope. The C8D3 heavy chain sequence can be SEQ ID NO: 154, and light chain sequence is SEQ ID NO: 155; the C8D3 CDRH1, CDRH2 and CDRH3 are SEQ ID NO: 156, 157 and 158, respectively, and CDRL1, CDRL2 and CDRL3 are SEQ ID NO: 159, 160 and 161, respectively. Also described in this publication are four hybridoma clones which produce antibodies to C3d (*i.e.*, Clones B7, C2, C6 and C8). The C6 heavy chain sequence is SEQ ID NO: 162, and light chain sequence is SEQ ID NO: 163; the C6 CDRH1, CDRH2 and CDRH3 are SEQ ID NO: 164, 165 and 166, respectively, and CDRL1, CDRL2 and CDRL3 are SEQ ID NO: 167, 168 and 169, respectively.

[0088] In some embodiments, the C3d antibody can be an antibody which binds C3d, but not C3c ("Neo-Anti-C3d"), as described in US Patent Application Publication No. 2015/0139899. The binding between the C3d antibody and its epitope with an affinity of about 100 pM to about 500 pM, *e.g.*, 447 pM. The C3d antibody can also be an antibody specific for the neoepitope iC3b ("Neo Anti-iC3b"), and, in some cases, bind its epitope with an affinity of about 100 pM to about 500 pM *e.g.*, 262 pM, however, said antibody does not bind C3c or C3d.

[0089] In some embodiments, the C3d antibody can be monoclonal antibody M130 which is specific for an antigenic determinant expressed by C3bi, C3dg, and C3d, which is almost undetectable in C3 and C3b. M130 has been shown to have higher affinity to C3d and iC3b than C3(H20) and can bind to C3d at residues 1209-1236, and 1217-1232.

[0090] In some embodiments, the C3d antibody can be monoclonal antibody C3-12.2 which binds to human, rat and mouse C3dg fragments. It was prepared using C3 deficient mice immunized with human C3b, iC3b and C3dg protein mixture, and has KD of about 95 nM measured by BiaCore. C3-12.2 and antibodies 3d29, 3d8b and 3d9a can recognize one or more overlapping C3 fragments or variants and the Fab appears to bind to the same or neighboring epitope as CR2.

[0091] In some embodiments, the C3d antibody can be monoclonal antibody 15-39-06 which was raised in wild-type rats using a synthetic peptide derived from human C3dg and conjugated to diphtheria toxin. It may have specificity to C3dg complement split product.

[0092] In some embodiments, the C3d antibody can be commercially available antibodies specific to C3d and/or other C3 fragments (*i.e.*, C3b, iC3b, C3c, C3dg, *etc.*). Examples

include antibodies available from Quidel, monoclonal antibodies A250 and A209 are reported to be specific for the neoepitopes C3d and iC3b, respectively. Antibody A250 was shown to agglutinate EC3bi, EC3b and EC3d cells in an indirect hemagglutination assay, and was also shown to bind to radio-labeled purified iC3b, C3b, and C3d but not to similarly labeled C3, or C3c. Antibody A209 was shown to agglutinate EC3bi but not EC3b or EC3d cells in an indirect hemagglutination assay, and was also shown to bind to radio-labeled purified iC3b but not to similarly labeled C3, C3b, C3d, or C3c. Further examples include available from Abeam are anti-C3d antibody 7C10 with unknown epitopes and anti-C3d antibody [E28-P] (abl36916) which is reported to bind an epitope at the N-terminus of C3d. In some cases, commercial antibodies can be antibodies available from BioRad are anti-C3d antibody 053 A- 514.3.1.4 and iC3b antibody 013 III-1.16 (f.k.a. MCA2607) are reported to be specific to neoantigens C3d and iC3b, respectively. Available from Sigma is antibody 3E7 is reported to recognize C3b and iC3b. Available from Origene, monoclonal antibody AM26358PU-N reacts with a neoantigen on iC3 (C3(H2O)), iC3b, C3dg and/or C3g and recognizes iC3b, C3dg and C3g in plasma but does not recognize C3 or C3b. Available from U.S. Biological Life Sciences is antibody C0010-19 rat anti-C3g (recognizing iC3, iC3b, C3dg). Also available from U.S. Biological Life Sciences, C7850-13V-ML550 mouse anti-complement C3b, inactivated. This antibody is reported to recognize human inactivated complement C3b (iC3b) neoantigen in blood serum. Available from Hycult are a number of antibodies including antibody HM2199, anti-human C3g mAb 9 (YB2/90-5-20, which reacts with a neoantigen on iC3, iC3b, C3dg and C3g, and which recognizes iC3b, C3dg and C3g in plasma but does not recognize C3 or C3b; monoclonal antibody HM2198 - anti-human C3d, mAb 3 (YB2/39-11-1-7) which is reported to react with a linear determinant in C3d found on C3, C3b, iC3b, C3dg and C3d and which recognizes C3, C3b, iC3b, C3dg and C3d, but not C3c; antibody HM2168 - anti-activated human C3, clone bH6 which is specific for a C3 neoepitope expressed on the cleavage fragments of C3b, iC3b and C3c, but not C3dg and C3f; and antibody HM2257 - activated human C3, mAb 13/15 which recognizes activated complement protein C3 or more specifically a neoepitope on C3b, iC3b and C3dg which are not present in native C3. Available from Meridian, antibody H54189M is reported to react with the alpha chain of C3b, but not with C3a or C3d, and allows demonstration of C3 deposits in tissue, on cells, on microorganisms and in immune complexes.

[0093] Each of the 3d9a, 3d29 and 3d8b antibodies may be able to bind to kidney tissue sections exhibiting inflammation when injected into mice intravenously, and bind to C3-opsonized zymosan, which is known to express iC3b but not C3b. Thus, these antibodies

may be able to distinguish between tissue bound fragment C3d and circulating native C3 and the fragment C3b. The C3 binding antibody or antigen-binding fragment thereof, can bind to C3d or C3dg from multiple species (species cross-reactive). The anti-C3d or anti-C3dg antibody or antigen-binding fragment thereof can bind to C3d or C3dg from at least one species selected from human, non-human mammals (*e.g.*, cynomolgus monkey or cynomolgus macaque, rhesus macaque, ape, baboon, chimpanzee, orangutan, or gorilla), rodents (*e.g.*, mouse, rat, hamster, Guinea pig, gerbil, or rabbit), cattle, sheep, goat, donkey, pig, dog, cat, horse, and camel. The anti-C3d or anti-C3dg antibody or antigen-binding fragment thereof can bind to cynomolgus macaque C3d or C3dg. The anti-C3d or anti-C3dg antibody or antigen-binding fragment thereof described herein binds to C3d or C3dg from at least two species selected from the above list.

[0094] The anti-C3d or anti-C3dg antibody or antigen-binding fragment thereof binds to both human and cynomolgus macaque C3d or C3dg. The anti-C3d or anti-C3dg antibody or an antigen-binding fragment thereof can be an antibody selected from: 3d8b, 3d9a, 3d29, 3d11, 3d31, 3d3, 3d15, 3d10, and 3d16. The anti-C3d or anti-C3dg antibody or an antigen-binding fragment thereof can be an antibody selected from: 3d9a, 3d29 and 3d8b. The anti-C3d or anti-C3dg antibody or an antigen-binding fragment thereof can be 3d29.

[0095] In some embodiments, the anti-C3d or anti-C3dg antibody, or antigen-binding fragment thereof, of the complement inhibitor constructs, can compete with CR2 for binding to C3d or C3dg. Such an antibody or antigen-binding fragment thereof can reduce the ability of a CR2 protein to bind to human complement component C3d or C3dg by greater than 50 (*e.g.*, greater than 55, 60, 65, 70, 75, 80, 85, 90, or 95 or more) %. For example, the CR2-C3d binding can be decreased to at least 60%, at least 40%, or to a % value in between. The anti-C3d or anti-C3dg antibody, or antigen-binding fragment thereof, can significantly inhibit or block CR2 binding to C3d. In some embodiments, such an antibody is 3d9a, 3d29 or 3d8b. Exemplary fusion protein constructs comprising anti-C3d or anti-C3dg targeting domains and a complement modulator, in some cases, can better compete with CR2 binding to C3d or C3dg, than an anti-C3d or anti-C3dg targeting domain alone.

[0096] The complement inhibitor constructs, comprising a targeting moiety and the complement modulator, can include an antibody or an antigen-binding fragment thereof as the targeting moiety. Examples of the targeting moiety include, but are not limited to, a monoclonal antibody or antibody fragment, a diabody, a chimerized or chimeric antibody or antibody fragment, a humanized antibody or antibody fragment, a deimmunized human antibody or antibody fragment, a fully human antibody or antibody fragment, a bispecific

antibody or antibody fragment, a monovalent antibody or antibody fragment, a single chain antibody, an immunoglobulin G1 (IgG1) heavy chain, a single chain variable fragment (*i.e.*, an scFv), sc(fv)₂, a tandem scFv, a diabody, a VHH domain, a VH domain, an Fv, an Fd, an Fab heavy chain, an Fab light chain, an Fab, an Fab', and Fab' light chain, an Fab' heavy chain, and an F(ab')₂. The antibody or antigen binding fragments that form the targeting moiety, can be a human antibody, a humanized antibody, a murine antibody.

[0097] In some embodiments, the antigen-binding fragment thereof includes: (i) Fab fragments; (ii) F(ab')₂ fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide.

[0098] In some embodiments, the antigen-binding fragment thereof is an Fab, Fab', F(ab')₂, Fd, single chain Fv or scFv, disulfide linked Fv, V-NAR domain, IgNar, intrabody, IgGACH2, minibody, F(ab')₃, tetrabody, triabody, diabody, single-domain antibody, DVD-Ig, Fcab, mAb₂, (scFv)₂, or scFv-Fc.

[0099] In some embodiments, the flexible peptide linker contains amino acid residues that provide flexibility, such as glycine, serine, alanine, or threonine. In some embodiments, the peptide linker has a length that is adequate to link two moieties in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. Suitable lengths for this purpose may include at least one to about 100 amino acid residues or more. In some embodiments, the linker is from about 1 to 30 amino acids in length. In some embodiments, the linker is from about 1 to 20 amino acids in length. In some embodiments, the peptide linker promotes proper protein folding, stability, expression, and/or bioactivity of the component protein moieties. In some embodiments, the flexible peptide linker is composed of glycine, serine, or threonine, with multiple glycine residues to provide a highly flexible conformation. Serine or threonine residues provide polar surface area to limit hydrophobic interaction within the peptide or with the component fusion protein moieties. The amino acid residues selected for inclusion in the linker peptide can exhibit properties that do not interfere significantly with the activity of the polypeptide. Thus, the linker peptide may not exhibit a charge that would be inconsistent with the activity of the polypeptide, or interfere with internal folding, or form bonds or other interactions with amino acid residues in the targeting moiety or the complement modulator that would seriously impede the binding of the moieties to their targets.

[0100] Non-limiting examples of sequences which may serve as the linker can include short peptides of about 2 to about 15 amino acids in length. Among the peptide sequences that can be used as linkers in this disclosure are (Gly-Ser)_n, where n = 0, 1, 2, 3, 4, 5, 6, 7, or 8 (SEQ ID NO: 292); (GlyGlyGlySer)_n, where n = 1, 2, 3, or 4 (SEQ ID NO: 293); (GlySerSerGly)_n, where n = 1, 2, 3, or 4 (SEQ ID NO: 294). In some embodiments, the linker sequence is GGGSGGGGS (SEQ ID NO: 138).

[0101] Glycine-alanine polymers, alanine-serine polymers, and other flexible linkers such as a tether blocker for the shaker potassium channel, comprising, a panel of quaternary ammoniums (QA) linked to maleimides with varying length poly-glycine tethers, and a large variety of other flexible linkers can also be used. Glycine-serine polymers can be used since both amino acids are relatively unstructured, and therefore may be able to serve as a neutral tether between components. Secondly, serine is hydrophilic and therefore able to solubilize what could be a globular glycine chain.

[0102] In some cases, the linker can comprise a sequence as set forth in any one of SEQ ID NOs: 171-193, where, in some examples, n can be at least 4; in some examples, n can be between 1 and 8, between 1 and 5. For instance, in SEQ ID NO: 161, n can be at least 4; in SEQ ID NO: 164, n = 1-8; in SEQ ID NO: 165, n = 1-5; in SEQ ID NO: 166, n = 1-5. In SEQ ID NO: 168, X can be A (alanine), K (lysine), or E (glutamic acid), and n = 5-17. In some cases, n in SEQ ID NOs.: 161, 164, 165, 166, 168, 170, 171, 174, and 179 can range from 1 to 17, 1 to 8, 1 to 5, at least 4, or 5 to 17.

[0103] Exemplary complement inhibitors described herein are provided for illustrative purpose only, and are by no means limiting.

[0104] In some embodiments, the complement inhibitor is a fusion protein construct comprising: 1) an antibody or an antigen binding fragment thereof that specifically binds to complement protein 3d (c3d), wherein the antibody or antigen binding fragment thereof comprises: (a) a heavy chain comprising three heavy chain complementarity determining regions (CDR-H1, CDR-H2, CDR-H3), wherein the CDR-H1 comprises the amino acid sequence of SEQ ID NO: 29, the CDR-H2 comprises the amino acid sequence of SEQ ID NO: 260, and the CDR-H3 comprises the amino acid sequence of SEQ ID NO: 31, and, (b) a light chain comprising three light chain complementarity determining regions (CDR-L1, CDR-L2, CDR-L3), wherein the CDR-L1 comprises the amino acid sequence of SEQ ID NO: 32, the CDR-L2 comprises the amino acid sequence of SEQ ID NO: 33, and the CDR-L3 comprises the amino acid sequence of SEQ ID NO: 34, and 2) a complement modulator

polypeptide, wherein the complement modulator polypeptide comprises factor H or a biologically active fragment thereof.

[0105] In some embodiments, the light chain comprises a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 258.

[0106] In certain embodiments, the light chain comprises the amino acid sequence of SEQ ID NO: 279.

[0107] In some embodiments, the heavy chain comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 254.

[0108] In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO: 284.

[0109] In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO: 282.

In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO: 285.

[0110] In some embodiments, in the fusion protein construct: (a) the light chain comprises a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 258; and, (b) the heavy chain comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 254.

[0111] In some embodiments, the light chain comprises the amino acid sequence of SEQ ID NO: 279.

[0112] In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO: 284.

[0113] In some embodiments, the fusion protein further comprises a linker linking the antibody or antigen binding fragment thereof to the complement modulator polypeptide.

[0114] In some embodiments, the linker comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 138, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, and SEQ ID NO: 241.

[0115] In some embodiments, the linker is bound to the C-terminus of the heavy chain and comprises the amino acid sequence of SEQ ID NO: 138.

[0116] In some embodiments, the complement modulator polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 72 and SEQ ID NO: 108.

[0117] In some embodiments, the complement modulator polypeptide comprises an amino acid sequence of SEQ ID NO: 72.

[0118] In some embodiments, the antibody or antigen binding fragment thereof comprises: (a) a first heavy chain and a second heavy chain, wherein each of the first and the second heavy chain comprises three heavy chain complementarity determining regions (CDR-H1, CDR-H2, CDR-H3), wherein the CDR-H1 comprises the amino acid sequence of SEQ ID NO: 29, the CDR-H2 comprises the amino acid sequence of SEQ ID NO: 260, and the CDR-H3 comprises the amino acid sequence of SEQ ID NO: 31, and (b) a first light chain and a second light chain, wherein each of the first and the second light chain comprises three light chain complementarity determining regions (CDR-L1, CDR-L2, CDR-L3), wherein the CDR-L1 comprises the amino acid sequence of SEQ ID NO: 32, the CDR-L2 comprises the amino acid sequence of SEQ ID NO: 33, and the CDR-L3 comprises the amino acid sequence of SEQ ID NO: 34.

[0119] In some embodiments, each of the first and the second heavy chain comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 254, and wherein each of the first and the second light chain comprises a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 258.

[0120] In some embodiments, the first and the second heavy chain each comprises the amino acid sequence of SEQ ID NO: 284, and wherein the first and the second light chain each comprises the amino acid sequence of SEQ ID NO: 279.

[0121] In some embodiments, the first and the second heavy chain each comprises the amino acid sequence of SEQ ID NO: 282.

[0122] In some embodiments, the first and the second heavy chain each comprises the amino acid sequence of SEQ ID NO: 285.

[0123] In some embodiments, the fusion protein further comprises a linker linking the antibody or antigen binding fragment thereof to one of the complement modulator polypeptides.

[0124] In some embodiments, the linker comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 138, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID

NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, and SEQ ID NO: 241.

[0125] In some embodiments, the fusion protein construct comprises: (c) a first linker bound to the C-terminus of the first heavy chain and comprising the amino acid sequence of SEQ ID NO: 138; and (d) a second linker bound to the C-terminus of the second heavy chain and comprising the amino acid sequence of SEQ ID NO: 138.

[0126] In some embodiments, the complement modulator polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 72 and SEQ ID NO: 108.

[0127] In some embodiments, the fusion protein construct is selected from the group consisting of: (1) two heavy chain-containing polypeptides, each comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 282, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 72; and, two light chain-containing polypeptides each comprising the amino acid sequence of SEQ ID NO: 279; (2) two heavy chain-containing polypeptides, each comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 285, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 72; and, two light chain-containing polypeptides each comprising the amino acid sequence of SEQ ID NO: 279; (3) a heavy chain-containing polypeptide comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 284, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 72; and, a light chain-containing polypeptide comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 279, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 72; (4) a heavy chain-containing polypeptide comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 284, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 72; and, a light chain comprising the amino acid sequence of SEQ ID NO: 279; (5) two heavy chain-containing polypeptides, each comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 282, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 108; and, two light chain-containing polypeptides each comprising the amino acid sequence of SEQ ID NO: 279; (6) two heavy chain-containing polypeptides, each comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 285, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 108; and, two light chain-containing polypeptides each comprising the amino acid sequence of SEQ ID

NO: 279; (7) a heavy chain-containing polypeptide comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 284, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 108; and, a light chain-containing polypeptide comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 279, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 108; and, (8) a heavy chain-containing polypeptide comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 284, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 108; and, a light chain comprising the amino acid sequence of SEQ ID NO: 279.

[0128] In some embodiments, the complement inhibitor is a fusion protein construct comprising: 1) an antibody or an antigen binding fragment thereof that specifically binds to complement protein 3d (c3d), wherein the antibody or antigen binding fragment thereof comprises: (a) a heavy chain comprising three heavy chain complementarity determining regions (CDR-H1, CDR-H2, CDR-H3), wherein the CDR-H1 comprises the amino acid sequence of SEQ ID NO: 29, the CDR-H2 comprises the amino acid sequence of SEQ ID NO: 260, and the CDR-H3 comprises the amino acid sequence of SEQ ID NO: 31, and, (b) a light chain comprising three light chain complementarity determining regions (CDR-L1, CDR-L2, CDR-L3), wherein the CDR-L1 comprises the amino acid sequence of SEQ ID NO: 32, the CDR-L2 comprises the amino acid sequence of SEQ ID NO: 33, and the CDR-L3 comprises the amino acid sequence of SEQ ID NO: 34, and 2) a complement modulator polypeptide, wherein the complement modulator polypeptide comprises CR1 or a biologically active fragment thereof.

[0129] In some embodiments, the light chain comprises a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 258.

[0130] In some embodiments, the light chain comprises the amino acid sequence of SEQ ID NO: 279.

[0131] In some embodiments, the heavy chain comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 254.

[0132] In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO: 284.

[0133] In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO: 282.

[0134] In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO: 285.

[0135] In some embodiments, in the fusion protein construct: (a) the light chain comprises a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 258; and, (b) the heavy chain comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 254.

[0136] In some embodiments, the light chain comprises the amino acid sequence of SEQ ID NO: 279.

[0137] In some embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO: 284.

[0138] In some embodiments, the fusion protein further comprises a linker linking the antibody or antigen binding fragment thereof to the complement modulator polypeptide.

[0139] In some embodiments, the linker comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 138, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, and SEQ ID NO: 241.

[0140] In some embodiments, the linker is bound to the C-terminus of the heavy chain and comprises the amino acid sequence of SEQ ID NO: 138.

[0141] In some embodiments, the complement modulator polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 91, and SEQ ID NO: 92.

[0142] In some embodiments, the complement modulator polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 41 and SEQ ID NO: 42.

[0143] In some embodiments, the antibody or antigen binding fragment thereof comprises: (a) a first heavy chain and a second heavy chain, wherein each of the first and the second heavy chain comprises three heavy chain complementarity determining regions (CDR-H1, CDR-H2, CDR-H3), wherein the CDR-H1 comprises the amino acid sequence of SEQ ID NO: 29, the CDR-H2 comprises the amino acid sequence of SEQ ID NO: 260, and the CDR-H3 comprises the amino acid sequence of SEQ ID NO: 31, and (b) a first light chain and a second light chain, wherein each of the first and the second light chain comprises three light chain complementarity determining regions (CDR-L1, CDR-L2, CDR-L3), wherein the CDR-L1 comprises the amino acid sequence of SEQ ID NO: 32, the CDR-L2 comprises the

amino acid sequence of SEQ ID NO: 33, and the CDR-L3 comprises the amino acid sequence of SEQ ID NO: 34.

[0144] In some embodiments, each of the first and the second heavy chain comprises a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 254, and wherein each of the first and the second light chain comprises a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 258.

[0145] In some embodiments, the first and the second heavy chain each comprises the amino acid sequence of SEQ ID NO: 284, and wherein the first and the second light chain each comprises the amino acid sequence of SEQ ID NO: 279.

[0146] In some embodiments, the first and the second heavy chain each comprises the amino acid sequence of SEQ ID NO: 282.

[0147] In some embodiments, the first and the second heavy chain each comprises the amino acid sequence of SEQ ID NO: 285.

[0148] In some embodiments, the fusion protein further comprises a linker linking the antibody or antigen binding fragment thereof to one of the complement modulator polypeptides.

[0149] In some embodiments, the linker comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 138, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, and SEQ ID NO: 241.

[0150] In some embodiments, the fusion protein construct comprises: (c) a first linker bound to the C-terminus of the first heavy chain and comprising the amino acid sequence of SEQ ID NO: 138; and (d) a second linker bound to the C-terminus of the second heavy chain and comprising the amino acid sequence of SEQ ID NO: 138.

[0151] In some embodiments, the complement modulator polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 91, and SEQ ID NO: 92.

[0152] In some embodiments, the fusion protein construct is selected from the group consisting of: (1) two heavy chain-containing polypeptides, each comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 282, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 41; and, two light chain-containing

polypeptides each comprising the amino acid sequence of SEQ ID NO: 279; (2) two heavy chain-containing polypeptides, each comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 285, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 41; and, two light chain-containing polypeptides each comprising the amino acid sequence of SEQ ID NO: 279; (3) a heavy chain-containing polypeptide comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 284, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 41; and, a light chain-containing polypeptide comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 279, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 41; (4) a heavy chain-containing polypeptide comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 284, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 41; and, a light chain comprising the amino acid sequence of SEQ ID NO: 279; (5) two heavy chain-containing polypeptides, each comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 282, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 42; and, two light chain-containing polypeptides each comprising the amino acid sequence of SEQ ID NO: 279; (6) two heavy chain-containing polypeptides, each comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 285, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 42; and, two light chain-containing polypeptides each comprising the amino acid sequence of SEQ ID NO: 279; (7) a heavy chain-containing polypeptide comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 284, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 42; and, a light chain-containing polypeptide comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 279, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 42; and, (8) a heavy chain-containing polypeptide comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 284, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 42; and, a light chain comprising the amino acid sequence of SEQ ID NO: 279.

[0153] Also provided is a method of treating a patient in need of reducing glomerular complement activity / activation, the method comprising administering a complement inhibitor to the patient upon confirming that the patient has an elevated normalized urinary C5b-9 (uC5b-9) level compared to a control / reference standard.

[0154] Numerous complement mediated or associated diseases or disorders affect the

kidney or have a kidney component. Thus, patients being treated for such complement mediated or associated diseases are in need of reducing glomerular complement activity.

[0155] In some embodiments, the variety of complement-associated diseases or disorders include, without limitation: ischemia-reperfusion injury, rheumatoid arthritis (RA); lupus nephritis; ischemia-reperfusion injury; atypical hemolytic uremic syndrome (aHUS); typical or infectious hemolytic uremic syndrome (tHUS); dense deposit disease (DDD); paroxysmal nocturnal hemoglobinuria (PNH); multiple sclerosis (MS); macular degeneration (e.g., age-related macular degeneration (AMD), geographic atrophy (also known as atrophic age-related macular degeneration or advanced dry AMD); hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome; sepsis; dermatomyositis; diabetic retinopathy; thrombotic thrombocytopenic purpura (TTP); spontaneous fetal loss; Pauci-immune vasculitis; epidermolysis bullosa; recurrent fetal loss; multiple sclerosis (MS); and traumatic brain injury.

[0156] In some embodiments, the complement-mediated vascular disorder can include 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-Schonlein purpura nephritis, systemic lupus erythematosus-associated vasculitis, vasculitis associated with rheumatoid arthritis, immune complex vasculitis, Takayasu's disease, capillary leak syndrome, dilated cardiomyopathy, diabetic angiopathy, thoracic-abdominal aortic aneurysm, Kawasaki's disease (arteritis), venous gas embolus (VGE), and restenosis following stent placement, rotational atherectomy, and percutaneous transluminal coronary angioplasty (PTCA).

[0157] In some embodiments, the complement-associated disorder can be myasthenia gravis, cold-agglutinin disease (CAD), paroxysmal cold hemoglobinuria (PCH), idiopathic inflammatory myopathies including dermatomyositis and polymyositis, scleroderma, warm autoimmune hemolytic anemia, Graves' disease, Hashimoto's thyroiditis, type I diabetes, psoriasis, pemphigus, autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), Goodpasture's syndrome, antiphospholipid syndrome (APS), Degos disease, and catastrophic APS (CAPS).

[0158] In some embodiments, ischemia-reperfusion (IR) injury can refer to damage to a tissue caused when the blood supply returns to the tissue after a period of ischemia (restriction in blood supply). The absence of oxygen and nutrients from the blood creates a

condition in which the restoration of circulation results in inflammation and oxidative damage, rather than restoration of normal function.

[0159] Ischemia-reperfusion injury can be associated with traumatic injury, including hemorrhagic shock, as well as many other medical conditions such as stroke or large vessel occlusion (*e.g.*, middle cerebral artery), and is a major medical problem. More particularly, ischemia-reperfusion injury is important in heart attacks, stroke, kidney failure following vascular surgery, post-transplantation injury and chronic rejection, as well as in various types of traumatic injury, where hemorrhage will lead to organ hypoperfusion, and then subsequent reperfusion injury during fluid resuscitation. Ischemia-reperfusion injury, or an injury due to reperfusion and ischemic events, is also observed in a variety of autoimmune and inflammatory diseases.

[0160] Independent of other factors, ischemia-reperfusion injury may lead to increased mortality. Ischemia-reperfusion injury, as well as hypovolemic shock and subsequent tissue damage, has been shown to be caused by complement and Fc receptor activation and the recruitment and activation of neutrophils and other inflammatory cells. It had also been shown that single monoclonal antibodies that react broadly with phospholipids and other extracellular or intracellular antigens such as DNA can cause ischemia-reperfusion injury in mice that lack other antibodies (*i.e.*, B cell- deficient mice).

[0161] Renal disease and symptoms thereof (such as albuminuria, or more broadly, proteinuria) can include abnormalities in kidney function that lead to increased levels of protein excretion in the urine. Increased protein in the urine is a marker of kidney injury or kidney damage caused by immune disorders, glomerulonephritis, multiple myeloma, cardiovascular diseases, or kidney trauma.

[0162] The ratio of albumin and creatinine present in the urine is typically used to detect potential kidney injury or disease. In some embodiments, the ratio of protein (including albumin) and creatinine present in the urine is referred to as uPCR (urinary protein creatinine ratio). Persistent elevated urine protein levels may be indicative of kidney injury or disease.

[0163] The ratio of uC5b-9 to urine creatinine (uC5b-9/uCr) or the ratio of uC5b-9 to uPCR (uC5b-9/uPCR) is a superior biomarker of tissue complement activation, as uPCR measures broader changes in kidney function, and therefore only indirectly reflects tissue complement activity.

[0164] Thus, in some embodiments, a uC5b-9/uCr or uC5b-9/uPCR ratio is determined. In some embodiments, a uC5b-9/uCr or uC5b-9/uPCR ratio is determined for a subject having a disease (*e.g.*, renal disease). In some embodiments, a uC5b-9/uCr or uC5b-9/uPCR

ratio is determined in a biological sample. In some embodiments, the biological sample is urine. In some embodiments, a uC5b-9/uCr or uC5b-9/uPCR ratio determined for a subject having a disease is compared to a uC5b-9/uCr or uC5b-9/uPCR ratio from another subject or the same subject (*e.g.*, another subject with the same disease, or the same subject at different time points, or another subject not having the disease). In some embodiments, the uC5b-9/uCr or uC5b-9/uPCR ratio is determined at some point after the subject is administered with any of the complement inhibitor construct of this disclosure. In some embodiments, the subject is administered with a comparable complement inhibitor construct. In some embodiments, the comparable complement inhibitor construct does not comprise the antibody or the antigen binding fragment thereof but is otherwise identical to a fusion protein complement inhibitor construct of this disclosure. In some embodiments, the uC5b-9/uCr or uC5b-9/uPCR ratio is determined from a biological sample (*e.g.*, urine sample) from a subject (*e.g.*, a subject having a disease). In some embodiments, the uC5b-9/uCr or uC5b-9/uPCR ratio from a subject having a disease is at least about 1%, 2%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% lower than the uC5b-9/uCr or uC5b-9/uPCR ratio from a comparable biological sample from the same subject collected at a different time point or from another subject (*e.g.*, another subject administered with a comparable fusion protein construct).

[0165] In certain instances, an anti-C3d/C3dg antibody or antigen-binding fragment thereof or a fusion complement inhibitor construct comprising such antibody or an antigen-binding fragment thereof as the targeting moiety described herein, alone or in combination with a second anti-inflammatory agent, can be used to treat an inflammatory disorder such as, but not limited to, RA (above), inflammatory bowel disease, sepsis (above), septic shock, acute lung injury, disseminated intravascular coagulation (DIC), or Crohn's disease. In some embodiments, the second anti-inflammatory agent can be one selected from NSAIDs, corticosteroids, methotrexate, hydroxychloroquine, anti-TNF agents such as etanercept and infliximab, a B cell depleting agent such as rituximab, an interleukin-1 antagonist, and a T cell costimulatory blocking agent such as abatacept.

[0166] In some embodiments, the complement-associated disorder is a complement-associated neurological disorder such as, but not limited to, amyotrophic lateral sclerosis (ALS), brain injury, Alzheimer's disease, and chronic inflammatory demyelinating neuropathy.

[0167] In some embodiments, the complement-associated disorder is complement-associated pulmonary disorders such as, but not limited to, asthma, bronchitis, a chronic

obstructive pulmonary disease (COPD), an interstitial lung disease, α -1 anti-trypsin deficiency, emphysema, bronchiectasis, bronchiolitis obliterans, alveolitis, sarcoidosis, pulmonary fibrosis, and collagen vascular disorders.

[0168] In some embodiments, the complement-associated disorder is: Paroxysmal nocturnal hemoglobinuria (PNH), Atypical hemolytic uremic syndrome (aHUS), myasthenia gravis, Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), C3 glomerulonephritis (C3G), cold agglutinin disease (CAD), warm antibody hemolytic anemia, antibody mediated transplant rejection, Neuromyelitis Optica (NMOSD), dense deposit disease, IgA Nephropathy (IgAN), Membranous Nephropathy (MN), thrombotic microangiopathy, hereditary angioedema (HAE), IgG4-related disease (IgG4 RD), ANCA-associated vasculitis (AAV), hypertensive nephropathy, diabetic nephropathy, thrombotic microangiopathy, systemic lupus, lupus nephritis, discoid lupus, psoriatic arthritis, psoriasis, atopic dermatitis, alopecia areata, hidradenitis suppurativa, vitiligo, rheumatoid arthritis, periodontal disease, bone disorders (osteoarthritis, fracture, osteomyelitis), Dry macular degeneration, Wet macular degeneration, glaucoma, uveitis, geographic atrophy, heart and lung surgery, cardiac regeneration, lung cancer, neurodegeneration, ALS, MS, traumatic brain injury, spinal cord injury, schizophrenia, major depressive disorder, bipolar disorder, scleroderma, scleroderma renal crisis, cutaneous vasculitis, blistering skin diseases (endemic pemphigus, bullous pemphigoid, Pemphigus erythematosus, pemphigus vulgaris), and drusen-related disease.

[0169] In some embodiments, the complement-mediated disease is a complement-mediated inflammation.

[0170] In some embodiments, the complement-mediated disease is characterized by an increased deposition of C3d.

[0171] In some embodiments, the complement-mediated disease is characterized by an increased deposition of C2 antibody -reactive phospholipid.

[0172] In some embodiments, the complement-mediated disease is complement-mediated inflammation, wherein the complement mediated inflammation comprises an inflammatory fibrotic disease, and wherein the inflammatory fibrotic disease comprises focal segmental glomerulosclerosis, primary sclerosing cholangitis, or membranoproliferative glomerulonephritis.

[0173] In some embodiments, the complement-mediated disease is a complement-mediated auto-immune disease, comprising rheumatoid arthritis, systemic lupus erythematosus, lupus nephritis, or pemphigus vulgaris.

[0174] In some embodiments, the complement-mediated disease is a complement-mediated kidney disease, comprising membranoproliferative glomerulonephritis, or complement 3 glomerulopathy.

[0175] In some embodiments, the complement-mediated disease is a complement-mediated cardiovascular disease. In some embodiments, the cardiovascular disease comprises atherosclerosis or thrombosis.

[0176] In some embodiments, the complement-mediated disease is a complement-mediated dermatological disease. In some embodiments, the dermatological disease comprises psoriasis, acne inversa, lupus erythematosus, cutaneous small vessel vasculitis, urticaria, urticarial vasculitis, or bullous pemphigoid.

[0177] In some embodiments, the complement-mediated disease is a complement-mediated inflammation, and wherein the complement-mediated inflammation is associated with a condition or disease selected from the group consisting of ischemia/reperfusion injury, burn injury, endotoxemia and septic shock, adult respiratory distress syndrome, cardiopulmonary bypass, hemodialysis, anaphylactic shock, asthma, angioedema, Crohn's disease, sickle cell anemia, glomerulonephritis, membranous nephritis, pancreatitis, transplant rejection, hyperacute xenograft rejection, recurrent fetal loss, preeclampsia, drug allergy, IL-2 induced vascular leakage syndrome, radiographic contrast media allergy, myasthenia gravis, Alzheimer's disease, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, insulin-dependent diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, antiphospholipid antibody syndrome, autoimmune hepatitis, Goodpasture's syndrome, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, idiopathic thrombocytopenic purpura, pemphigus, Sjogren's syndrome, Takayasu's arteritis, myocardial infarction, stroke, acute respiratory distress syndrome, sepsis, plasmapheresis, plateletpheresis, leukopheresis, extracorporeal membrane oxygenation, heparin-induced extracorporeal LDL precipitation, bowel inflammation, urticarial, vasculitis, and lupus nephritis.

[0178] In some embodiments, the complement-mediated disease is selected from the group consisting of ischemia-reperfusion injury, rheumatoid arthritis (RA), lupus nephritis, ischemia-reperfusion injury, atypical hemolytic uremic syndrome (aHUS), typical or infectious hemolytic uremic syndrome (tHUS), dense deposit disease (DDD), paroxysmal nocturnal hemoglobinuria (PNH), multiple sclerosis (MS), macular degeneration, hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, sepsis, dermatomyositis, diabetic retinopathy, thrombotic thrombocytopenic purpura (TTP), spontaneous fetal loss,

Pauci-immune vasculitis, epidermolysis bullosa, recurrent fetal loss, multiple sclerosis (MS), traumatic brain injury, a cardiovascular disorder, myocarditis, a cerebrovascular disorder, a peripheral vascular disorder, a renovascular disorder, a mesenteric/enteric vascular disorder, revascularization to transplants and/or replants, vasculitis, Henoch-Schonlein purpura nephritis, systemic lupus erythematosus-associated vasculitis, vasculitis associated with rheumatoid arthritis, immune complex vasculitis, Takayasu's disease, capillary leak syndrome, dilated cardiomyopathy, diabetic angiopathy, thoracic-abdominal aortic aneurysm, Kawasaki's disease (arteritis), venous gas embolus (VGE), and restenosis following stent placement, rotational atherectomy, percutaneous transluminal coronary angioplasty (PTCA), myasthenia gravis, cold-agglutinin disease (CAD), paroxysmal cold hemoglobinuria (PCH), dermatomyositis, scleroderma, warm autoimmune hemolytic anemia, Graves' disease, Hashimoto's thyroiditis, type I diabetes, psoriasis, pemphigus, autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), Goodpasture's syndrome, antiphospholipid syndrome (APS), Degos disease, and catastrophic APS (CAPS).

[0179] In some embodiments, the complement-mediated disease is selected from the group consisting of age-related macular degeneration (AMD), type II membranoproliferative glomerulonephritis (MPGN II), hemolytic uremic syndrome (HUS), asthma, amyloidosis, and thrombotic thrombocytopenic purpura. In some embodiments, the hemolytic uremic syndrome (HUS) is an atypical hemolytic uremic syndrome (aHUS).

[0180] In some embodiments, the complement-mediated disease is a drusen-associated disease or a drusen-related disease. In some embodiments, the drusen-related disease is amyloidosis, elastosis, dense deposit disease, glomerulonephritis, atherosclerosis or an ocular drusen-related disease.

[0181] While preferred embodiments of this disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from this disclosure. It should be understood that various alternatives to the embodiments of this disclosure described herein may be employed in practicing the disclosure. It is intended that the claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Sequences

[0182] All nucleic acid and amino acid sequences referred to herein as SEQ ID NO: X are identical to the sequences with the same SEQ ID NO described in PCT/US2019/065741 (published as WO/2020/123662 on 6-18-2020), which sequences including its sequence listing are incorporated herein by reference.

[0183] For example, “SEQ ID NO: 282” referred to herein is identical to SEQ ID NO: 282 of WO/2020/123662, and has the following sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFNTYYINWVRQAPGQGLEWMGVINPYSGGTSYNQKFKGRV
TMTVDSTSTAYMELSSLRSEDTAVYFCSSPYWGQGLTVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDPKPSNTK
VDKRVESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDG
VEVHNAKTKPREEQFNSTYRVVSVLTVQLHQLDNLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVLHEALHSHYTQKSLSLSLG

[0184] “SEQ ID NO: 279” referred to herein is identical to SEQ ID NO: 279 of WO/2020/123662, and has the following sequence:

DVMTQSPFLSLPVTLGQPASISCKSSQSLDSDGKTYLNWFQQRPGQSPRRLLIYLVSKLDSGVPDRFS
GSGSGTDFTLKISRVEADVGVYCWQGTHFPRTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

[0185] “SEQ ID NO: 72” referred to herein is identical to SEQ ID NO: 72 of WO/2020/123662, and has the following sequence:

EDCNELPPRRNTEILTGSDQTYPEGTQAIYKCRPGYRSLGNVIMVCRKGEWVALNPLRKCQKRPCG
HPGDTFPFTLTGGNVFEYGVKAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPEN
GKIVSSAMEPDREYHFQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDVINGSPIS
QKIIYKENERFYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSPLRIKHRTGDEI
TYQCRNGFYPATRGNTAKCTSTGWIPAPRCTLK

[0186] “SEQ ID NO: 41” referred to herein is identical to SEQ ID NO: 41 of WO/2020/123662, and has the following sequence:

QCNAPFWLPFARPTNLTDEFEPPIGTLYLNYECRPGYSGRPFSSIICLNKSVWTGAKDRCRKSCRNPPD
PVNGMVHVIKGIQFGSQIKYSCTKGYRLIGSSSATCIISGDTVWDNETPICDRIPCGLPPTITNGDF
ISTNRENPHYGSVVTYRCNPGSGGRKVFELVGEPISYCTSNDDQVGIWSPAPQCIIPNKCTPPNVEN
GILVSDNRSLSLNEVVEFRCPGPFVMKGPRRVKCAALNKWEPELPSCSRVCQPPPDVLHAERTQRDK
DNFSPGQEVFYSCPEGYDLRGAASMRCTPQGDWSPAAPTCEVKSCDDFMGQLLNGRVLFVNLQLGAK
VDFVCDGEFQLKGSSASYCVLAGMESLNSSVPVCEQIFCPSPPVIPNGRHTGKPLEVFPFGKTVNYT

CDPHPDRGTSFDLIGESTIRCTSDPQNGVWSSPAPRCGILGHCQAPDHFLFAKLKTQTNASDFPIGT
 SLKYECPREYYGRPFSTICLDNLVWSSPKDVCKRKSCKTPDPVNGMVHVITDIQVGSRINYSCTTGH
 RLIGHSSAECILSGNAAHWSTKPPICQRIPCGLPPTIANGDFISTNRENPHYGSVVTYRCNPGSGGRK
 VFELVGEPSTYCTSNDDQVGIWSGPAPQCIIPNK

[0187] “SEQ ID NO: 138” referred to herein is identical to SEQ ID NO: 138 of
 WO/2020/123662, and has the following sequence:

GGGSGGGGS

[0188] “SEQ ID NO: 29” referred to herein is identical to SEQ ID NO: 29 of
 WO/2020/123662, and has the following sequence:

GYTFTNYY

[0189] “SEQ ID NO: 260” referred to herein is identical to SEQ ID NO: 260 of
 WO/2020/123662, and has the following sequence:

INPYSGGT

[0190] “SEQ ID NO: 31” referred to herein is identical to SEQ ID NO: 31 of
 WO/2020/123662, and has the following sequence:

SSPY

[0191] “SEQ ID NO: 32” referred to herein is identical to SEQ ID NO: 32 of
 WO/2020/123662, and has the following sequence:

QSLDSDGKTY

[0192] “SEQ ID NO: 33” referred to herein is identical to SEQ ID NO: 33 of
 WO/2020/123662, and has the following sequence:

LVS

[0193] “SEQ ID NO: 34” referred to herein is identical to SEQ ID NO: 34 of
 WO/2020/123662, and has the following sequence:

WQGTHFPRT

[0194] “SEQ ID NO: 258” referred to herein is identical to SEQ ID NO: 258 of
 WO/2020/123662, and has the following sequence:

DVVMTQSPLSLPVTLGQPASISCKSSQSLDSDGKTYLNWFQQRPGQSPRRLIYLVSKLDSGVPDRFS
 GSGSGTDFTLKISRVEAEDVGYYCWQGTHFPRTFGGGTKVEIK

[0195] “SEQ ID NO: 254” referred to herein is identical to SEQ ID NO: 254 of
 WO/2020/123662, and has the following sequence:

QVQLVQSGAEVKKPGASVKVSKASGYTFTNYYINWVRQAPGQGLEWMGVINPYSGGTSYNQKFGRV
 TMTVDSTSTAYMELSSLRSEDVAVYFCSSPYWGQGLTVTVSS

[0196] “SEQ ID NO: 284” referred to herein is identical to SEQ ID NO: 284 of
 WO/2020/123662, and has the following sequence:

QVQLVQSGAEVKKPGASVKVSCASGYTFTNYYINWVRQAPGQGLEWMGVINPYSGGTSYNQKFGRV
TMTVDTSTSTAYMELSSLRSEDTAVYFCSSPYWGQGLTVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTTYTCNVDPKPSNTK
VDKRVESKYG

[0197] “SEQ ID NO: 285” referred to herein is identical to SEQ ID NO: 285 of WO/2020/123662, and has the following sequence:

QVQLVQSGAEVKKPGASVKVSCASGYTFTNYYINWVRQAPGQGLEWMGVINPYSGGTSYNQKFGRV
TMTVDTSTSTAYMELSSLRSEDTAVYFCSSPYWGQGLTVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTTYTCNVDPKPSNTK
VDKRVESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDG
VEVHNAKTKPREEQFNSTYRVVSVLTVQLHQLDNLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMHEALHNHYTQKSLSLGLG

[0198] Likewise, “SEQ ID NOs: 7-22, 42, 91, 92, 108, 154-193, 241, 292-294” referred to herein is identical to SEQ ID NOs: 7-22, 42, 91, 92, 108, 154-193, 241, 292-294, respectively, of WO/2020/123662, and all such sequences have been incorporated herein by reference.

EXAMPLES

Example 1 C3d Deposition in Human Renal Diseases

[0199] Both urine and plasma C3d have been identified as biomarkers in renal diseases including C3 glomerulopathy (C3G) and systemic lupus erythematosus (SLE). Anti-C3c immunostaining of kidney biopsies are also clinically relevant, but C3d tissue deposition in human disease is less well characterized.

[0200] Here, tissue from a subset of kidney diseases was surveyed, using the mouse anti-C3d antibody 3d8b to detect C3d deposit in human renal tissues.

[0201] Strong C3d immunofluorescence was detected in renal biopsies from patients with C3G (data not shown), consistent with the well-established role for complement in this disease. Low-to-moderate C3d immunostaining was detected in samples from patients diagnosed with thrombotic microangiopathy (data not shown), anti-neutrophilic cytoplasmic autoantibody (ANCA) vasculitis (data not shown), and antibody-mediated rejection (AMR) of transplanted kidney (data not shown). Stronger immunostaining was evident in samples from membranous glomerulonephropathy (MGN) (data not shown), IgA nephropathy (IgAN) (data not shown), and both class III and class IV lupus (data not shown). Semi-quantitative

scoring of anti-C3d immunostaining confirmed generally higher C3d deposition in C3G, MGN, IgAN, and both classes of lupus nephritis (data not shown). These findings are also broadly consistent with similar quantitation of anti-C3 fragment (C3c), which is commonly used for clinical evaluation of kidney biopsies (data not shown).

[0202] To further explore the correlation between complement activity in selected renal diseases, a retrospective analysis of C3 fragment staining in a larger sample cohort was performed. Consistent with the prospective analysis, these data revealed a significant number of ANCA patients (43/104) that were C3 fragment-positive, while a patients MGN (86.2% of samples positive), IgA (89.7% of samples positive), and lupus nephritis (96.3% of class III and 87.0% of class IV samples positive) revealed a high prevalence of glomerular complement activation in these diseases.

[0203] Taken together, these data in kidney suggest broad translational potential of C3d-targeting as a means of locally delivering a complement inhibitor across multiple autoimmune indications and target organs.

Example 2 Generation and in vitro Characterization of Human and Mouse C3d-targeted fH₁₋₅

[0204] A few anti-C3d-targeted fH₁₋₅ fusion proteins, collectively designated C3d-mAb-2fH, were generated to localize complement inhibition to C3d-positive tissue.

[0205] Mouse and human C3d-mAb-2fH are approximately 213 kDa recombinant bifunctional fusion proteins consisting of an anti-C3d monoclonal antibody linked to two moieties of the first five short consensus repeats (SCRs) of factor H (fH₁₋₅). See FIG. 1. Anti-C3d targeting of mouse C3d-mAb-2fH (ADX-118) and the mouse/human chimeric fusion ADX-048 both rely on the monoclonal mouse IgG1 antibody 3d8b, which binds with low nM affinity to an epitope present in mouse, cynomolgus monkey, and human C3d, iC3b, and C3dg. The 3d8b antibody was subsequently humanized by CDR grafting onto a human germline acceptor framework, followed by additional amino acid modifications to improve antibody stability and minimize concerns of antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). The resulting human IgG4 antibody, ADX-093, retained similar affinity as 3d8b to human C3d and was used in the human C3d-mAb-2fH fusion protein, ADX-097.

[0206] C3d-mAb-2fH antibody fusions inhibit AP complement via the first 5 of 20 short complement regulator domains (SCRs) of fH (fH₁₋₅), which are necessary and sufficient to

catalyze AP convertase dissociation by accelerating decay of the C3bBb complex and inactivating it by cleavage of C3b to iC3b.

[0207] ADX-118 incorporates mouse fH₁₋₅, while ADX-048 and ADX-097 use the equivalent human sequence. It had been shown that fusion of complement regulatory domains to the C-termini of a C3d targeting antibody heavy chain yielded optimal complement inhibition. Therefore, in mouse, human, and chimeric fusion proteins, a fH₁₋₅ moiety was linked to each heavy chain C-terminus via (Gly₄Ser)₂ linkers (see FIG. 1). Fusion of fH₁₋₅ did not affect anti-C3d antibody binding, as ADX-118 and ADX-097 binding affinities to mouse, cynomolgus monkey, and human C3d are similar to their parent antibodies, 3d8b and ADX-093 (data not shown).

[0208] *In vitro* complement inhibition of ADX-097 was evaluated by measuring the formation of AP- or CP-generated C5b-9 (MAC) in human complement-preserved serum (Wieslab assays, Svar Life Sciences). Consistent with its role in AP regulation, human recombinant fH₁₋₅ inhibited AP complement (IC₅₀ = 1980 ± 163 nM) but demonstrated no measurable inhibition of CP complement. Fusion of two fH₁₋₅ moieties to a human Fc domain (ADX-145) demonstrated a roughly six-fold increase in AP complement inhibition (IC₅₀ = 325 ± 61 nM), suggesting a potential avidity effect conferred by the presence of a second fH₁₋₅ domain. This is consistent with prior studies using an engineered dimeric fH₁₋₅ fusion protein. The humanized anti-C3d antibody ADX-093 by itself showed no inhibition of AP complement, but fusion of two fH₁₋₅ domains (ADX-097) resulted in a further four-fold increase in potency vs. ADX-145 (IC₅₀ = 81 ± 3.9 nM). ADX-097 retained strong selectivity for AP complement, as inhibition of CP-initiated complement activity, though measurable, is approximately 20-fold weaker (IC₅₀ = 1545 ± 42 nM). A chimeric molecule consisting of two moieties of human fH₁₋₅ linked to mouse 3d8b (ADX-048) showed similar AP complement inhibition as ADX-097 (IC₅₀ = 73 ± 8.3 nM), indicating that fusion of fH₁₋₅ to either the mouse or human anti-C3d antibodies results in similar potency.

[0209] Wieslab AP and CP assays are designed to work in human serum but do not work well using rodent sera. Therefore, to assess C3d-mAb-2fH fusion proteins' potency in mouse and rat, a crucial prerequisite for *in vivo* testing in rodent species, an assay that relies on zymosan particles incubated in complement preserved serum was used. The human fusion protein ADX-097 showed similar potency in human and mouse serum (IC₅₀ = 191 ± 17 nM in human and 202 ± 54 nM in mouse) and was slightly more potent in rat serum (IC₅₀ = 99 ± 17 nM). In comparison to ADX-097, the mouse fusion protein ADX-118 was 3-4x more potent

in mouse ($IC_{50} = 46 \pm 3.9$) but 2-3x less potent in rat serum (281 ± 52 nM). ADX-118 showed no inhibitory activity in human serum.

[0210] Together, these data demonstrate activity of both the mouse and human fusion proteins in rodent, enabling subsequent *in vivo* studies to assess tissue-targeted pharmacokinetics and pharmacodynamics (PK/PD).

[0211] At low C3d densities that favor monovalent binding, the C3d-targeting monoclonal antibodies (mAbs) (ADX-093 and ADX-058/3d8b) and the human C3d-mAb-2fH mAb fusion protein (ADX-097) exhibited similar binding properties to the Fabs (data not shown). However, as C3d density increased, mAb and mAb-fusion affinity improved substantially. Increasing C3d density 420-fold, for example, led to a ~2000x increase in k_d compared to binding of monovalent Fab fragments (data not shown). This improved binding at high densities is due to prolonged k_{off} 's, suggesting that it is driven primarily through mAb avidity. As a consequence, under conditions that approximate the high-density C3d deposition observed in complement-active tissue, ADX-097 exhibits low picomolar binding affinity, representing a 10,000-100,000-fold increase over TT30 (a single moiety of fH fused to the iC3b-, C3dg-, and C3d-ligand binding domain of the B-cell receptor CR2 (CD21)).

[0212] These data suggest that, compared to CR2 targeting, both improved Fab affinity and avidity of the bivalent targeting antibody may confer substantial improvements in both targeting efficiency and durability.

Example 3 Evaluation of C3d-mAb-2fH Activity on Human Skin Explants

[0213] This example demonstrates inhibition of complement in the context of human tissue.

[0214] Human C3d-mAb-2fH (ADX-097) was evaluated in a skin explant assay. Specifically, cryosections of human skin were pre-incubated with normal human serum or serum from BP patients under conditions that prevent complement activation and deposition, allowing pathogenic anti-BP180 antibodies in the BP serum to bind the skin section. After washing, sections were then incubated with complement-active human serum (+/- inhibitors) and complement deposition detected by immunofluorescence with an anti-C3b antibody.

[0215] Sections pre-incubated with normal serum exhibit minimal anti-C3b immunofluorescence, while those pre-incubated with BP serum show substantial C3b deposition on the tissue (data not shown). Addition of the anti-C3d binding antibody ADX-093 to the complement-active serum incubation had no effect, while ADX-097 concentrations

as low as 0.28 μ M significantly inhibited skin C3b deposition ($P < 0.0002$, data not shown), with complete inhibition of complement occurring between 0.28 and 1.4 μ M (60-300 μ g/ml ADX-097 in serum). These data indicate that ADX-097 can inhibit human tissue complement deposition, even in the highly dysregulated context of BP serum.

Example 4 *C3d-mAb-2fH Localizes to Active Complement in Primate Skin*

[0216] This example demonstrates that human C3d-mAb-2fH (ADX-097) can be targeted to local complement *in vivo*.

[0217] High-dose UV-B irradiation has been shown to induce epidermal complement activation. Thus, a model of UV-B-induced skin complement activation was developed in cynomolgus monkeys. Specifically, erythema was induced in monkey skin by transient exposure to high doses of UV-B light using a hand-held lamp. Skin biopsies were then collected at intervals after exposure and immunostained using anti-C3c and anti-C3d antibodies (data not shown). Colocalized deposition of complement fragments was observed in the epidermis as early as 24 hours after exposure and lasted for at least 72h (data not shown).

[0218] To show localization of C3d-mAb-2fH to primate skin, UV-B injury was induced on study day -1, 24 hours prior to drug dosing. Human C3d-mAb-2fH was administered systemically by SC injection on day 0, and blood and tissue biopsies were collected daily for one week after dosing. Complement activation was observed after UV-B exposure in vehicle (PBS)-treated monkeys (data not shown). Skin localization was detected as early as 24 hours after dosing, and total tissue drug exposure, measured as area under the curve (AUC), was greater than the PBS control for all doses tested ($P < 0.02$) (data not shown). Both circulating and tissue drug exposure were dose-correlated: 10 mg/kg ADX-097 showed greater tissue drug accumulation than the 1 mg/kg dose ($P < 0.05$), though the difference between the 10 and 30 mg/kg groups' tissue drug localization was not statistically significant (data not shown). In the 10 and 30 mg/kg dose groups, inhibition of circulating complement appears to wane as circulating drug levels drop below approximately 70 μ g/mL (data not shown). Notably, while ADX-097 localizes to tissue in the 1 mg/kg dose group, circulating drug concentration in this group remains below 10 μ g/mL throughout the study, indicating no inhibition of systemic complement in this group.

[0219] All tested doses of ADX-097 showed similar tissue complement inhibition, reaching maximal inhibition roughly 3 days after SC dosing (data not shown). However, this

difference was not statistically significant ($P = 0.11$ to 0.15), a likely consequence of the small number of samples ($n = 3$) collected at each time point.

[0220] Nevertheless, these data in a non-human primate skin model demonstrate that ADX-097 is capable of localizing to tissue *in vivo* and may locally inhibit complement at doses that do not affect the systemic complement.

Example 5 C3d-targeted fH₁₋₅ Localized to Complement-active Tissue in Mouse and Blocked Tissue Complement without Systemic Complement Inhibition

[0221] To further demonstrate C3d-mAb-2fH *in vivo* tissue targeting and local complement inhibition, a series of pharmacology studies were carried out in fH knockout mice (CfH^{-/-}). Due to the loss of functional fH protein, CfH^{-/-} mice exhibit elevated complement activation in liver and kidney and sporadically develop complement mediated renal injury.

[0222] Three days after a single 50 mg/kg intravenous (IV) dose of chimeric C3d-mAb-2fH (ADX-048) or controls, kidney and liver tissue were collected for evaluating complement activity by fluorescent immunostaining for active C3 split products (C3b/iC3b/C3c, which were collectively referred to as “anti-C3 fragment”) and drug distribution by staining with anti-human fH (anti-fH). Anti-C3 fragment immunostaining was stronger in knockout renal glomeruli and liver than in wild-type mice (data not shown) and only background anti-fH immunofluorescence was detected in liver or kidney from either WT mice or CfH^{-/-} mice treated with PBS (data not shown). In CfH^{-/-} mice dosed with untargeted human fH₁₋₅ (hufH₁₋₅), anti-fH immunostaining also indicated no hufH₁₋₅ localization to liver or kidney (data not shown) and complement activity in liver and renal glomeruli was also unchanged compared to CfH^{-/-} + PBS (data not shown), indicating that non-targeted inhibition was insufficient to block tissue AP complement at this time point.

[0223] In contrast, significant anti-fH immunostaining was evident in CfH^{-/-} mice treated with a single dose of ADX-048 (data not shown), demonstrating fusion protein targeting to tissues with high levels of active complement. Furthermore, these mice showed a marked decrease in both liver and kidney anti-C3 fragment immunofluorescence (data not shown), indicating that, unlike non-targeted hufH₁₋₅, C3d-mAb-2fH potently inhibits complement activity in tissue for at least a week after dosing.

[0224] Tissue kinetics of C3d-mAb-2fH-mediated glomerular complement inhibition were then assessed using lower doses of C3d-mAb-2fH. Kidneys were collected from CfH^{-/-} mice after IV dosing with 5 mg/kg human C3d-mAb-2fH (ADX-097), with 5 mg/kg C3d

targeting antibody alone (ADX-093), or with vehicle control. Glomerular immunofluorescence in anti-C3 fragment-stained kidney sections was then digitally quantified to measure tissue complement inhibition. Glomerular C3 fragment deposition in ADX-093-dosed $CfH^{-/-}$ mice was in the same range as in untreated $CfH^{-/-}$ mice (data not shown). However, dosing with ADX-097 significantly and durably reduced glomerular C3 fragment deposition in $CfH^{-/-}$ mice (data not shown). Immunofluorescence using a human anti-IgG4 antibody demonstrated homing of both the ADX-093 and ADX-097 proteins to glomeruli (data not shown). Furthermore, the ADX-097 fusion protein appears to remain intact *in vivo*, as detection with both anti-fH and anti-IgG4 show similar tissue distribution (data not shown). These data demonstrate that both the human and human/mouse chimeric C3d-mAb-2fH fusion proteins localize to and block tissue convertase activity in $CfH^{-/-}$ mice and that this inhibition requires the presence of fH moieties on the molecule.

[0225] A time course study in $CfH^{-/-}$ mice more thoroughly evaluated potency of C3d-mAb-2fH and characterized the relationship between circulating and tissue PK/PD. These studies relied on the mouse C3d-mAb-2fH, ADX-118, to minimize the potential for anti-drug antibody (ADA) formation that could affect drug exposure. ADX-118 was dosed subcutaneously (SC) at doses ranging from 0.3 to 25 mg/kg and IV at 5 mg/kg. Plasma and tissue were collected at multiple time points and tissue complement and drug localization were detected by anti-C3 fragment and anti-fH immunostaining (data not shown). Glomerular immunofluorescence from 3-4 animals per time point was then quantified from digital images. All doses resulted in glomerular localization of ADX-118 (data not shown), with the tissue C_{max} correlating with drug dose. Localized drug was detected in tissue for at least 10 days in all tested doses, returning to background levels by 14-17 days post-dose. Comparison of SC vs. IV dosing at 5 mg/kg suggests more rapid drug distribution to tissue after IV dosing that translated to a difference in tissue complement inhibition at the earliest time point assessed (8 hours), while at all other time points, complement inhibition achieved by SC vs. IV dosing was similar (data not shown).

[0226] In contrast to the dose-dependence of tissue drug exposure, maximal complement inhibition was achieved in the 1, 5, and 25 mg/kg ADX-118 dose groups, though lower doses required a longer time to reach maximal inhibition (data not shown). In fact, maximum tissue complement inhibition in the 1 and 5 mg/kg SC groups was reached prior to tissue C_{max} (data not shown), suggesting that saturation of anti-C3d target binding is not required to fully inhibit tissue AP complement.

[0227] Complement inhibition was observed in the 0.3 mg/kg dose group but did not reach the maximum inhibition achieved in the 1, 5, and 25 mg/kg dose groups (data not shown). Finally, one week after dosing, the 1, 5 and 25 mg/kg groups all retained maximal complement inhibition, indicating that ADX-118-mediated tissue complement inhibition is quite durable.

[0228] In addition to localized complement activity in tissue, CfH^{-/-} mice also exhibit a 10 to 20-fold reduction in circulating intact C3 protein levels, as uncontrolled AP complement activity consumes C3 faster than new C3 protein is generated. In vivo administration of exogenous AP complement inhibitor temporarily reduces this C3 consumption, leading to a transient increase in intact plasma C3 that can serve as a sensitive biomarker of systemic complement inhibition. Twenty-four hours after delivery, a single 25 mg/kg SC dose of ADX-118 reached a circulating C_{max} of approximately 50 µg/mL (data not shown). This corresponded to a measurable increase in intact plasma C3 (data not shown), indicating partial inhibition of systemic complement that returned to baseline as drug cleared from circulation. IV delivery of 5 mg/kg ADX-118 also resulted in sufficient drug exposure (~30 µg/mL) to elicit transient elevation of intact plasma C3 over the course of 24 hours (data not shown). In contrast, SC delivery of ≤ 5 mg/kg ADX-118 resulted in negligible plasma C3 elevation, consistent with the lower C_{max} and overall exposure levels at these doses (data not shown).

[0229] Thus, while SC doses of 1-5 mg/kg are capable of potent and durable local inhibition in tissue, they do not achieve sufficiently high circulating drug concentrations to affect systemic complement in CfH^{-/-} mice.

Example 6 C3d-targeted fH1-5 Reduced Renal Injury in a Rat Model of Membranous Nephropathy

[0230] The example demonstrates the disease-modifying efficacy of C3d-mAb-2fH in the Passive Heymann Nephritis (PHN) model of membranous nephropathy in rat.

[0231] PHN is induced by administering a sheep serum raised against a preparation of rat proximal tubules (anti-Fx1A). See FIG. 2A. Injection of anti-Fx1A induces subepithelial immune deposits in the glomerular basement membrane (GBM), leading to pathologic changes in the GBM and podocytes that are reflected as elevated urine protein levels. Renal injury in PHN is driven by complement activation, as treatment with cobra venom factor (CVF), a C3 analog that depletes endogenous complement, or with a small molecule factor B inhibitor, effectively reduces disease in this model.

[0232] Because C3d-mAb-2fH targeting occurs through C3d binding, the time-course of C3d deposition after anti-Fx1A treatment was first assessed (FIG. 2B). Consistent with the role of complement in PHN, glomerular anti-C3d immunostaining was first detected in kidneys two days after delivery of anti-Fx1A (FIG. 2B) while proteinuria, measured as urine protein/creatinine ratio (uPCR), appeared by day 3 ($P < 0.001$ vs healthy control, FIG. 2C). Based on these data, C3d-mAb-2fH was dosed on day 3 to evaluate therapeutic efficacy after disease onset.

[0233] The human C3d-mAb-2fH, ADX-097, was initially tested via IV administration at doses ranging from 1 to 30 mg/kg. As a comparator, a separate cohort of rats was dosed daily with 100 U/kg CVF beginning two days prior to disease induction (see study design, FIG. 2A). The human drug candidate ADX-097 was used in these studies because the short drug-treatment period (2-4 days) minimizes concerns that ADAs could affect drug exposure.

[0234] ADX-097 administered after onset of proteinuria (day 3 after disease induction) reduced progression of proteinuria (uPCR) as early as 24 hours after injection, and to a similar degree as prophylactic CVF treatment (FIG. 2C). This was not due to diminished anti-Fx1A localization, as equivalent sheep IgG deposition was detected in ADX-097-treated and control PHN glomeruli collected five days after disease induction (2 days after ADX-097 dosing) (data not shown). No correlation between ADX-097 dose and reduced uPCR progression was observed, suggesting maximal efficacy at the lowest tested dose (1 mg/kg) and indicating that ADX-097 potentially inhibits renal injury in PHN.

[0235] However, a non-targeted control molecule with two moieties of fH₁₋₅ fused to an Fc domain (Fc-2fH₁₋₅) also significantly reduced proteinuria progression when administered at a dose equimolar to 30 mg/kg ADX-097, raising the possibility that at high doses, C3d targeting is dispensable for ADX-097 efficacy in this model.

[0236] To further explore local effects of ADX-097 in diseased tissue, both glomerular and systemic complement activity were examined in samples collected two days after ADX-097 administration (Day 5 after disease induction). In PHN kidney tissue, ADX 097 treatment led to dose-dependent reduction in glomerular complement activity (anti-C3 fragment immunostaining) (FIG. 2D). Notably, while ADX-097 doses ≥ 10 mg/kg completely inhibited activity, the 1 and 3 mg/kg dose groups showed approximately 40% and 75% reduction in C3 fragment deposition, respectively.

[0237] Paired with the reduction in uPCR described above, these data suggest that partial complement inhibition may be sufficient for disease-modifying efficacy. In accordance with its effects on proteinuria, non-targeted Fc-2fH₁₋₅ also inhibited glomerular C3 fragment

deposition (FIG. 2D). However, while both the 30 mg/kg ADX-097 and Fc-2fH₁₋₅ groups had similar concentrations in circulation (FIG. 2E), only ADX-097 localizes to glomeruli (FIG. 2G), indicating that Fc-2fH₁₋₅ efficacy at this high dose is mediated through fluid-phase/systemic complement inhibition. Consistent with this hypothesis, both Fc-2fH₁₋₅ and 30 mg/kg ADX-097 also inhibited serum complement (FIG. 2F). In contrast, no systemic complement inhibition was detected in samples from animals treated with 1, 3, or 10 mg/kg ADX-097 (FIG. 2F), indicating that C3d targeting drives the potency of ADX-097's effects on complement-mediated disease at these lower doses.

[0238] The C5b-9 protein complex is an end-product of complement activation, leading to formation of pores that disrupt pathogen and target cell membranes, leading to cell lysis and death. Deposition of C5b-9 is also a tissue marker of complement activity that can be detected in a wide spectrum of kidney diseases, including membranous, IgA, hypertensive, and diabetic nephropathies, lupus nephritis, thrombotic microangiopathies, and C3 glomerulopathy. Soluble C5b-9 is also detected in urine (uC5b-9) from patients suffering from IgAN, membranous nephropathy, and preeclampsia and in the rat PHN model, suggesting that soluble uC5b-9 may reflect tissue complement activity in the kidney.

[0239] Because ADX-097 inhibits complement in tissue without affecting circulating complement, the PHN model provides an opportunity to further evaluate soluble uC5b-9 as an indicator of renal complement. In urine samples collected from PHN rats on study day 5, 48 hours after treatment with 1, 3, or 10 mg/kg ADX-097 or with PBS (see FIG. 2A), uC5b-9 concentration, normalized to urine Creatinine (uC5b-9/uCre) was reduced by ADX-097 in a dose-dependent manner (FIG. 3A). Furthermore, a highly significant correlation (Spearman $r = 0.76$; $P < 0.00000001$) between uC5b-9/uCre and tissue glomerular C3 fragment deposition, measured by anti-C3 fragment immunostaining, was found (FIG. 3B), suggesting that uC5b-9/uCre is a urine biomarker that closely reflects kidney tissue complement activation.

[0240] Similarly, uC5b-9 concentration, normalized to uPCR (uC5b-9/uPCR) was similarly reduced by ADX-097 in a dose-dependent manner (FIG. 3C). Furthermore, a highly significant correlation between uC5b-9/uPCR and tissue glomerular C3 fragment deposition, measured by anti-C3 fragment immunostaining, was also found (FIG. 3D), suggesting that uC5b-9/uPCR is also a urine biomarker that closely reflects kidney tissue complement activation.

[0241] In a follow-on study, ADX-097 potency in the PHN model was further explored using lower doses of ADX-097 (0.3, 1, and 3 mg/kg) to understand the minimum efficacious

dose (see study design, FIG. 4A). ADX-097 was delivered by SC injection to reduce the circulating drug C_{max} , further testing the role of C3d-mediated tissue targeting. Because studies in $CfH^{-/-}$ mice indicated slower tissue distribution after SC delivery, an additional two days was added onto the study before collecting kidneys for analysis (4 days after ADX-097, 7 days after disease induction with anti-Fx1A). SC doses of Fc-2fH₁₋₅ at molar-equivalent doses to ADX-097 (0.17, 0.51, and 1.7 mg/kg, matching 0.3, 1, and 3 mg/kg ADX-097 respectively) to more directly compare C3d targeted vs. non-targeted complement inhibition.

[0242] At 48 hours after SC injection (day 5 after disease induction), 1 and 3 mg/kg doses of ADX-097 reduced uPCR relative to anti-Fx1A + PBS controls ($P < 0.0007$), while the 0.3 mg/kg ADX-097 group exhibited a non-statistically significant trend ($P = 0.07$) towards proteinuria reduction (FIG. 4B). By 96 hours after injection, all ADX-097 treatment groups exhibited a statistically significant difference in proteinuria ($P < 0.005$) that correlated with ADX-097 dose. A similar correlation was evident when uPCR was analyzed as area under the curve (uPCRAUC): Relative to anti-Fx1A + PBS, 3 mg/kg ADX-097 reduced uPCRAUC by $73 \pm 8.7\%$ ($P < 0.003$) and 1 mg/kg (SC) reduced uPCRAUC by $59 \pm 10\%$ ($P < 0.01$) (FIG. 4C). uPCRAUC reduction in the 0.3 mg/kg group ($33 \pm 22\%$) was not statistically significant, with the larger error reflecting a more variable response at this low dose. Efficacy of 3 mg/kg ADX-097 was similar to that of prophylactic CVF treatment (uPCR reduced by $76 \pm 11\%$). However, despite daily dosing with CVF, the reduction of uPCR in this group waned between study day 5 and 6, consistent with CVF's propensity to induce a neutralizing ADA response. This hypothesis is further supported by urine C5b-9/Cr, which was shown in the previous PHN study to closely correlate with tissue complement (FIGs. 3A and 3B). uC5b-9/uCr in the CVF treatment group was similar to non-diseased control rats until study day 5 but this effect was lost by study day 7 (FIG. 4D).

[0243] Similar to FIGs. 3A-3B, uC5b-9 concentration, normalized to uCr (uC5b-9/uCr) was reduced by ADX-097 in a dose-dependent manner (FIG. 5A). Furthermore, a highly significant correlation between uC5b-9/uCr and tissue glomerular C3 fragment deposition, measured by anti-C3 fragment immunostaining, was found (FIG. 5B), confirming that uC5b-9/uCr is a urine biomarker that closely reflects kidney tissue complement activation.

[0244] Similar to FIGs. 3C-3D, uC5b-9 concentration, normalized to uPCR (uC5b-9/uPCR) was reduced by ADX-097 in a dose-dependent manner (FIG. 5C). Furthermore, a highly significant correlation between uC5b-9/uPCR and tissue glomerular C3 fragment deposition, measured by anti-C3 fragment immunostaining, was found (FIG. 5D), confirming

that uC5b-9/uPCR is also a urine biomarker that closely reflects kidney tissue complement activation.

[0245] This study verified that the correlation between uC5b9 (normalized against uCr or uPCR) and glomerular C3, initially observed in PHN study depicted in FIG. 2A is replicated in a separate study with different timepoints, with SC dosing, and with different degrees of proteinuria at analyzed timepoint, as depicted in FIGs. 4A and 4B.

[0246] Based on these studies, Spearman correlation matrix in FIGs. 6A (for PHN study depicted in FIG. 2A) and 6B (for PHN study depicted in FIG. 4A) showed a closer correlation between either the uC5b9/uPCR or the uC5b9/uCr ratio and glomerular C3 fragment immunostaining than between uPCR and glomerular C3, indication that uC5b9/uPCR and uC5b9/uCr are superior to uPCR as urinary biomarkers of glomerular complement activity.

[0247] Specifically, in FIG. 6A, uPCR somewhat correlates to glomerular C3 (correlation coefficient $r = 0.39$, $P = 0.03$). Meanwhile, uC5b-9/uCr showed the strongest correlation to glomerular C3 ($r = \mathbf{0.76}$, $P = 3.8e-07$). uC5b-9/uCr also showed strong correlation to uPCR ($r = 0.60$, $P = 0.0003$). Similarly, uC5b9/uPCR also shows strong correlation to glomerular C3 ($r = \mathbf{0.68}$, $P = 0.00002$).

[0248] Similar observations were made in FIG. 6B: uPCR somewhat correlates to glomerular C3 (correlation coefficient $r = 0.41$, $P = 0.008$). Meanwhile, uC5b-9/uCr showed strong correlation to glomerular C3 ($r = \mathbf{0.60}$, $P = 0.001$), and uC5b9/uPCR also showed strong correlation to glomerular C3 ($r = \mathbf{0.55}$, $P = 0.0004$).

[0249] A subset of renal cortex samples collected on study day 7 were further analyzed by transmission electron microscopy (TEM) to compare proteinuria with ultrastructural changes in the glomeruli. Representative TEM from a healthy control glomerulus showed a glomerular basement membrane (GBM) of uniform thickness and displayed a distinct lamina densa. Healthy podocytes reside along the length of the GBM, with normal foot process morphology and well differentiated slit diaphragms). In contrast, representative glomeruli from rats treated with anti-FX1A exhibited extensive podocyte foot process effacement (data not shown), with rare slit diaphragm-like structures evident along a distorted GBM of varying thickness. Electron-dense subepithelial deposits were visible between some podocytes and the underlying GBM (data not shown), consistent with prior observations in the PHN model and likely representing accumulation of immune complexes at the filtration barrier.

[0250] In a PHN rat treated with 3 mg/kg ADX-097, ADX-097 rescued podocyte architecture along a substantial portion of the GBM (FIGs. 7A-7F). While local examples of

effaced podocytes and electron-dense deposits were observed, the number of well differentiated podocyte foot processes was clearly increased throughout multiple evaluated samples. This is consistent with the podocyte ultrastructure images obtained using super resolution confocal microscopy of Nephryn-immunostained kidney sections, as shown in FIGs. 8A-8C.

[0251] Together, these data further demonstrate both potent, dose-dependent attenuation of uPCR and preservation of glomerular ultrastructure after ADX-097 treatment.

[0252] To further understand the relationship between potency and tissue targeting in this study, ADX-097 efficacy was compared to equimolar doses of Fc-2fH₁₋₅. Forty-eight hours after SC delivery, 3 mg/kg ADX-097 and 1.7 mg/kg Fc-2fH₁₋₅ equivalently reduced uPCR relative to controls, though by 96 hours after dosing the reduction in the ADX-097 group was greater than in Fc-2fH₁₋₅-treated rats ($P < 0.05$) (data not shown). These data suggest that both proteins inhibit renal injury immediately after injection, but that efficacy of the C3d-targeted molecule is more durable as disease progresses. Comparison of the 1 mg/kg ADX-097 (SC and IV) and 0.57 mg/kg Fc-2fH₁₋₅ treatment groups revealed an even clearer efficacy difference, as ADX-097 but not Fc-2fH₁₋₅ reduced uPCR and uPCRAUC (data not shown). A similar difference was observed between the 0.3 mg/kg ADX-097 and 0.17 mg/kg Fc-2fH₁₋₅ treatment groups, though the difference between these low-dose groups is not statistically significant (data not shown).

[0253] The difference in efficacy between targeted and untargeted fusion proteins is also reflected in complement activity in tissue collected at end of study (day 7 after anti-Fx1A, day 4 after fusion protein delivery). While statistically significant reduction in complement anti-C3 fragment immunofluorescence was evident in glomeruli from rats injected SC with 1 or 3 mg/kg ADX-097, treatment with equimolar doses of Fc-2fH₁₋₅ had no statistically significant effect (data not shown). Also noted was that CVF did not inhibit tissue complement at 7 days, consistent with the hypothesis that CVF exposure was reduced due to ADA. Anti-fH immunostaining was detected in ADX-097-treated kidneys collected at 7 days, but not in those that received Fc-2fH₁₋₅ (data not shown), confirming lack of fH₁₋₅ localization in the absence of the C3d targeting antibody. No inhibition of systemic complement was seen in end-of-study serum samples collected from either the ADX-097 or Fc-2fH₁₋₅ treatment groups (data not shown). However, in urine collected as early as 24-hours after dosing, urine C5b-9/Cre ratio was reduced in the 1.7 mg/kg Fc-2fH₁₋₅-treated group (FIG. 4D), suggesting inhibition of systemic complement in the first 24-48 hours after dosing.

[0254] Also noted was 22- to 43-fold higher circulating exposure of Fc-2fH₁₋₅ compared to equimolar doses of ADX-097 (data not shown), indicating that C3d binding shortens the circulating half-life of C3d-mAb-2fH. A consequence of this would be greater systemic exposure of the Fc-2fH₁₋₅ fusion, potentially explaining the reduction in proteinuria observed in the 1.7 mg/kg Fc-2fH₁₋₅ dosing group (FIG. 4E). These data are, however, consistent with findings from the earlier PHN study (FIGs. 2F and 2G), indicating that circulating exposures of a fH₁₋₅-containing fusion protein ≤ 69 nM (14.9 μ g/ml) do not inhibit systemic complement activity.

[0255] Thus, comparing ADX-097 efficacy to that of equimolar doses of a non-targeted Fc-2fH₁₋₅ demonstrates that C3d-targeting contributes to the potency and durability of ADX-097 and enables inhibition of glomerular complement at doses that do not affect systemic complement activation.

[0256] The materials and methods used in the examples herein are provided below solely for illustrative purpose, and are not limiting in any respect.

Methods and Materials

Sourcing, immunostaining, and semiquantitative scoring of human samples

[0257] Frozen human kidney biopsy tissue blocks were supplied by Arkana Laboratories (Little Rock, AR). Kidney tissue cryosections were stained with anti-C3d mAb (clone 3d8b, mouse IgG₁ kappa) followed by a secondary FITC-conjugated goat anti-mouse IgG Fc (#115-095-205 Jackson ImmunoResearch, West Grove, PA). The fluorescence intensity of glomerular C3d staining was scored blinded by a pathologist for each case using a 0-3+ scale (negative staining was assigned as “0” and trace staining as “0.5” in the plotted graphs). A subset of the human kidney samples used in this study was stained for C3 deposition using a FITC-conjugated goat anti-human C3 polyclonal antibody (#B1C/B1A, Kent Labs, Bellingham, WA). The fluorescence intensity of glomerular C3 staining was scored blinded by a pathologist for each case using a 0-3+ scale (negative staining was assigned as “0” and trace staining as “0.5” in the graphs). A retrospective analysis was conducted based on the historical C3 scores to determine the frequency of C3 positive staining.

Humanization of 3d8b

[0258] The C3d targeting antibody for fusion proteins was humanized from mAb 3d8b using modeled structure-based complementarity-determining region (CDR) grafting into human germline gene acceptor frameworks.

Generation and expression of fusion proteins

[0259] Anti-C3d parental antibodies, antibody, Fab, and CR2 fusions, and Fc-fusions were transiently transfected in CHO cells using standard methods. Proteins were affinity purified over Protein A (Cytiva, Marlborough, MA) and buffer exchanged into phosphate buffered saline (PBS) pH 7.4 using size exclusion chromatography to yield material with greater than 95% purity and endotoxin levels below 0.5 EU/mg. Proteins were concentration by centrifugation through high flow polyethersulfone (PES) membranes and sterile filtered through 0.2 μ m filters.

Characterization of C3d binding

[0260] Binding affinity measurements were performed on Biacore 3000 or T200 at 25°C. A CM5 (carboxymethylated dextran) chip surface was equilibrated as per manufacturer protocol (Cytiva, Marlborough, MA) in 10 mM HEPES, 150 mM NaCl, 3 mM EDTA and 0.05% v/v Surfactant P20 (HBS-EP+). Flow cell 2, 3 and 4 of the CM5 chip were coated with low surface density of human, cynomolgus monkey, or mouse C3d using a standard EDC/NHS amine coupling method in sodium acetate pH 5.0. Flow cell 1 was activated and left blank as a reference channel. All flow cells were blocked with 1M ethanolamine to block any unoccupied binding sites. Anti-C3d antibodies and fusion proteins were diluted in HBS-EP+ running buffer in a concentration series ranging between 0-200 nM and injected over surface bound target antigen at a flow rate of 30 μ L/min for 120 s followed by dissociation in running buffer for 180 s. Binding of antibody fusion proteins was monitored in real time and fit using Langmuir (1:1) binding model. From the observed k_{on} and k_{off} , K_D was determined. Surfaces were regenerated using two injections of glycine at pH 1.7 for 40 s.

In vitro measurement of complement inhibition

[0261] Wieslab assays - Complement activation assays were performed using the Wieslab® AP and CP ELISAs (SVAR Life Science AB, Malmö, Sweden) according to manufacturer's instructions.

Complement deposition on human skin explants

[0262] Complement activation by BP immune complexes was performed as described. In brief, cryosections of human skin were incubated with normal human serum (negative control) or BP patient sera (positive control) diluted in PBS for 30 min at room temperature. To ensure that complement activation/deposition does not occur during this step, 10 mM EDTA was added. After washing twice for 10 min in PBS, normal human plasma (prepared from fresh- frozen plasma) diluted 1:5 in veronal buffer (Sigma Aldrich, UK) was added to

the slides and incubated for 30 min at room temperature. Subsequently, after two washing steps for 10 min in PBS, C3b deposition was detected by FITC-conjugated antibodies in PBS for 30 min (Dako #F0201 FITC- rabbit anti-human C3c). Experiments were carried out in presence of compounds 1-3 at 4 different concentrations. Compounds or a solvent were added with the addition of the complement source (diluted in patient serum and normal human plasma). Slides were examined by fluorescence microscopy and image analysis by a person not aware of the sections' treatments. Results are based on n=10/group, whereby the donor skin and patient IgG were varied. Statistical analysis was performed using SigmaPlot (Version 13). One-Way ANOVA was used to compare for differences among the groups. Dunnett's Method, comparing all experimental groups against positive control (BP IgG) was used as post-test.

UVB-induced skin complement activation model in non-human primates

[0263] NHP UVB skin model was performed at Biomere Inc. (Worcester, MA). Twenty 24 naïve cynomolgus monkeys of 2-5 years of age were enrolled. Animals were sedated with Telazol and UVB exposure at 3120 mJ/cm² was applied to dorsal skin on Day -1 using a SolRx 100-series Ultraviolet Phototherapy Lamp Unit (SolarcSystems, ON, Canada). A piece of UV light blocking film with a center hole of 2-cm in diameter was attached to the lamp unit surface to block all the UV light except the center 2-cm diameter area, so that only an area of 2-cm circle was exposed to UVB at each skin testing site. Each animal received a subcutaneous administration of ADX-097 or vehicle on Day 0. Blood and skin biopsies were collected at specified timepoints (Fig. 3A). Skin biopsies from naïve animals were collected at the time of the final, group-specific timepoint. All animals were returned to the testing facility's colony at the completion of post-biopsy medication and observations.

NHP Circulating PK/PD assays

[0264] Plasma PK analysis was conducted using an ADX-097-specific ELISA. Plates were coated with an anti-ideotype antibody that recognizes ADX-097 (Q32Bio CL00027) for total drug capture. Detection was accomplished using a biotinylated mouse anti-human factor H (ThermoFisher MA5-17735, clone OX-24) followed by a standard streptavidin HRP A/TMB colorimetric assay. Plasma complement activity assays were performed at Q32Bio using the Wieslab® AP Kit (SVAR Life Science AB, Malmö, Sweden) according to manufacturer's instructions. AP pathway activation was determined by normalizing values at each timepoint to pre-dose control.

NHP skin Immunostaining and image analysis

[0265] 5 mm frozen skin sections were stained after fixation with cold acetone at -20°C. Slides were rinsed with DPBS and loaded onto a Leica BOND Rx autostainer. ADX-097 tissue drug level was detected using a biotinylated mouse anti-human factor H primary antibody (ThermoFisher MA5-17735, clone OX-24) followed by detection with Alexa647-conjugated streptavidin (ThermoFisher S-21374). Slides were co-stained for complement fragment C3c using a FITC- rabbit anti-human C3c (DAKO F0201) antibody. Stained slides were mounted with VectaShield Vibrance anti-fade mounting media containing DAPI (VectorLab H-1800). Whole slide images were acquired using DAPI, FITC, and Cy5 channels with identical exposure times in each channel across all slides. VisioPharm software was used to identify tissue edges and tissue-free areas in order to define a regions-of-interest (ROI) corresponding to the epidermis. C3c and C3d signal in this ROI was quantified as an average signal intensity in the appropriate fluorescence channel.

CfH^{-/-} mice

[0266] C57BL/6 fH-deficient (*CfH^{-/-}*) mice carrying a targeted disruption of the gene encoding fH were generously provided by Prof. Matthew Pickering. Mice were housed and studies were conducted at Istituto Di Ricerche Farmacologiche Mario Negri (Italy) or at Biomere (Worcester, MA) according to internal institutional guidelines.

Mouse tissue immunostaining

[0267] Mouse or rat tissue samples were frozen in OCT. 5 µm cryosections were fixed in -20°C acetone. Rodent C3 fragments were detected using a FITC-conjugated goat anti-mouse C3 fragment polyclonal antibody (MPBiomedicals, 0855510) or a FITC-conjugated goat anti-rat C3 fragment polyclonal antibody (MPBiomedicals, 0855751). Rodent C3d was stained using a human anti-C3d IgG4 (clone 3d8b, ADX-086) followed by a secondary Alexa Fluor 647-conjugated mouse anti-human IgG4 pFc' (SouthernBiotech 9190-31 clone HP6023) or Alexa Fluor 488-conjugated mouse anti-human IgG4 pFc' (SouthernBiotech 9190-30 clone HP6023). Humanized C3d-mAb-2fH (ADX-097) was detected using a FITC-conjugated OX-24 (ThermoFisher MA5-17736). Mouse C3d-mAb-2fH (ADX-118) was detected using a FITC-conjugated mouse anti-mouse fH₁₋₄ monoclonal antibody (clone 2A5, a gift from Dr Claire Harris). Immunofluorescence quantitation was performed using an EVOS M5000 imaging system (ThermoFisher, Waltham, MA) and ImageJ software. A minimum of ten glomeruli were assessed per section.

Measurement of plasma C3 in CfH^{-/-} mice

[0268] Mouse blood was collected by cardiac puncture in the presence of EDTA, chilled on ice and plasma separated by centrifugation at 2000 g at 4 C within 15 min of collection. Mouse C3 was detected using a mouse C3 ELISA Kit (Genway Biotech, San Diego, CA GWB-7555C7) following manufacturer's instructions.

Plasma drug exposure assays (C3d-mAb-2fH and Fc-2fH)

[0269] C3d-mAb-2fH proteins in plasma were measured by ELISA. Technical details are available in the Supplemental Methods.

Passive Heymann Nephritis model of membranous nephropathy

[0270] Passive Heymann Nephritis (PHN) studies were performed at Inotiv Westminster (formerly Plato BioPharma, Inc.). 6-weeks old Male Sprague Dawley rats were obtained from Charles River Laboratories and allowed to acclimate for 5 days prior to study initiation. Rats were housed in metabolic cages throughout the study, starting at day 3. Nephritis was induced by administration of two doses of a sheep anti-Fx1A antibody (Dr. David Salant, Boston University School of Medicine, Boston, MA) delivered IV at 100 and 300 mg/kg on days 0 and 1 of the study respectively. Healthy control animals were dosed IV with normal sheep serum (Millipore, Burlington, MA). The positive control group was treated IP on day -1 with 150 U/kg Cobra Venom Factor, CVF (Quidel, San Diego, CA), then 100 U/kg daily from day 0 through end of study. All other study animals were treated IP with PBS on the same schedule. Test proteins ADX-097 and Fc-2fH₁₋₅ or PBS were delivered either SC or IV on day 3. Urine was collected from day 2 to day 7 and body weights and other physiological parameters were assessed daily. At study termination, serum and plasma were collected and both kidneys were collected for histology and immunofluorescence. For electron microscopy, two 1 mm short axis sections were harvested from one kidney pole, then cut into 8-10 cubes which were then immersion fixed in 2% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer (Electron Microscopy Sciences, Hatfield, PA) overnight at 4 C. Tissue cubes were then washed 3x in 0.1 mol/L sodium cacodylate buffer, 15 min per wash, then stored at 4 C in 0.1 mol/L sodium cacodylate buffer. Urinary protein and creatinine were measured on an Olympus AU400e Clinical Chemistry Analyzer (Beckman Coulter, Inc., Brea, CA) using clinical chemistry reagents: Micro-Total Protein and Creatinine (Sekisui Diagnostics, Burlington, MA). Urinary albumin was measured by ELISA, using a rat albumin-specific ELISA kit (Nephurat®, Ethos Biosciences, Inc, Logan Township, NJ).

Measurement of complement activity in rat serum

[0271] Blood was clotted at room temperature in serum separator tubes, incubated at room temperature, centrifuged, and stored at -80°C. Complement activity was measured using a modified zymosan assay protocol: 10 µl of serum was dispensed into duplicate wells of a 96 well V bottom plate containing 30 µl PBS + 0.1 % BSA buffer. Complement preserved rat serum (Complement Technologies, Tyler, TX) and serum with 10 mM EDTA served as positive and negative controls for each plate. 8.3% pre-activated zymosan (Complement Technologies, Tyler, TX), 16.6 mM EGTA, and 8.3 mM MgCl₂ in 0.1% BSA/PBS was added to all wells (final volume, 100 µl). After incubation, the complement reaction was quenched with 20 µl 50 mM EDTA and goat anti rat C3-FITC (MP Biomedicals, Solon, OH) was added. Following washing, the pellets were resuspended in PBS, pH 7.4, 0.1% BSA. Data were acquired on an Attune flow cytometer (ThermoFisher, Waltham, MA) collecting 10,000 events per well in autosampler mode, and were analysed in FlowJo (FlowJo, LLC, Ashland, OR). Median MFI values were measured to determine complement activity in the serum.

Measurement of soluble C5b-9 in rat urine

[0272] Test samples were assayed for soluble C5b-9 a Hycult TCC (Terminal Complement Complex) (HK-106) according to manufacturer's instructions, with the exception that measurement standard was diluted in diluent plus 25% male Sprague-Dawley urine (BioIVT# RAT00URINE0104496 Lot#RAT515719).

Transmission Electron Microscopy

[0273] Kidney tissue was fixed with 1% osmium tetroxide in 0.15M cacodylate buffer, dehydrated in an acetone series, and embedded in epoxy resin. Ultrathin sections were cut at 72 nm with a diamond knife, mounted on 200-mesh copper grids, and stained with 4% uranyl acetate and 0.4% lead citrate. Prepared sections were examined in a JEOL JEM-1010 transmission electron microscope, and digital images were collected at a range of magnifications with an Erlangshen ES100W digital camera (Gatan, Pleasanton, CA).

Statistics

[0274] Statistical significance was determined by one-way ANOVA unless otherwise specified. All statistical calculations were made using embedded functions in GraphPad Prism software.

CLAIMS:

1. A method of treating a patient in need of reducing glomerular complement activity / activation, the method comprising administering a complement inhibitor to the patient upon confirming that the patient has an elevated normalized urinary C5b-9 (uC5b-9) level compared to a control / reference standard.
2. The method of claim 1, wherein the normalized urinary C5b-9 (uC5b-9) level in the patient is normalized against uCr (urinary Creatinine) and/or uPCR (urinary Protein to Creatinine Ratio).
3. The method of claim 1 or 2, wherein the patient has a complement-mediated disease having complement activity / activation in kidney.
4. The method of claim 3, wherein the complement-mediated disease is focal segmental glomerulosclerosis, glomerulonephritis, complement 3 glomerulopathy (C3G), membranoproliferative glomerulonephritis, C3 glomerulonephritis, type II membranoproliferative glomerulonephritis (MPGN II), membranous nephropathy (MN), IgA nephropathy (IgAN), hypertensive nephropathy, diabetic nephropathy, thrombotic microangiopathy, lupus nephritis (LN), hemolytic uremic syndrome (HUS), atypical hemolytic uremic syndrome (aHUS), diabetic retinopathy, IgG4-related disease (IgG4 RD), or ANCA-associated vasculitis (AAV).
5. The method of claim 3, wherein the complement-mediated disease is AAV, IgAN, lupus nephritis (LN), or C3G.
6. The method of any one of claims 1-5, wherein the complement inhibitor is a tissue-specific complement inhibitor (*e.g.*, not a systemic complement inhibitor).
7. The method of claim 6, wherein the complement inhibitor specifically targets kidney.
8. The method of any one of claims 1-7, wherein the complement inhibitor comprises a binding moiety specific for C3 deposit in a disease tissue.
9. The method of claim 8, wherein the binding moiety is an antibody or an antigen-binding portion thereof specific for C3d.
10. The method of any one of claims 1-9, wherein the complement inhibitor comprises factor H, CR1, DAF, MCP, Crry, MAp44, MAp19, CD59, or a biologically active fragment thereof.

11. The method of any one of claims 1-10, wherein the complement inhibitor comprises:
 - (i) two heavy chain-containing polypeptides, each comprising, from N- to C-terminal, the amino acid sequence of SEQ ID NO: 282, the amino acid sequence of SEQ ID NO: 138, and the amino acid sequence of SEQ ID NO: 72 or 41; and,
 - (ii) two light chain-containing polypeptides, each comprising the amino acid sequence of SEQ ID NO: 279.
12. The method of any one of claims 1-11, further comprising ceasing or discontinuing treatment (*e.g.*, ceasing or discontinuing administering the complement inhibitor to the patient) upon confirming that the patient has normal level of normalized urinary C5b-9 (uC5b-9) compared to the control / reference standard.
13. The method of claim 12, wherein the patient has elevated normalized uPCR at the time of stopping / discontinuing treatment.
14. A method of identifying a patient as a candidate for treatment to reduce glomerular complement activity / activation, the method comprising determining a normalized urinary C5b-9 (uC5b-9) level in a urine sample from the patient, wherein an elevated level of the normalized urinary C5b-9 level, as compared to a control / reference standard, identifies the patient as the candidate in need of treatment to reduce glomerular complement activity / activation.
15. The method of claim 14, wherein the normalized urinary C5b-9 (uC5b-9) level in the patient is normalized against uCr (urinary Creatinine) and/or uPCR (urinary Protein to Creatinine Ratio).
16. The method of claim 14 or 15, wherein the patient has, or is at risk of having, a complement-mediated disease having complement activity / activation in kidney.
17. The method of claim 16, wherein the complement-mediated disease is focal segmental glomerulosclerosis, glomerulonephritis, complement 3 glomerulopathy (C3G), membranoproliferative glomerulonephritis, C3 glomerulonephritis, type II membranoproliferative glomerulonephritis (MPGN II), membranous nephropathy (MN), IgA nephropathy (IgAN), hypertensive nephropathy, diabetic nephropathy, thrombotic microangiopathy, lupus nephritis (LN), hemolytic uremic syndrome (HUS), atypical hemolytic uremic syndrome (aHUS), diabetic retinopathy, IgG4-related disease (IgG4 RD), or ANCA-associated vasculitis (AAV).

18. The method of claim 16, wherein the complement-mediated disease is AAV, IgAN, lupus nephritis (LN), or C3G.
19. A method of adjusting treatment of a patient in need of reducing glomerular complement activity / activation, wherein the treatment comprises administering a complement inhibitor to the patient, the method comprising: adjusting a dose and/or a frequency of administration of the complement inhibitor, based on the extent the patient has an elevated normalized urinary C5b-9 (uC5b-9) level compared to a control / reference standard.
20. The method of claim 19, wherein the normalized urinary C5b-9 (uC5b-9) level in the patient is normalized against uCr (urinary Creatinine) and/or uPCR (urinary Protein to Creatinine Ratio).