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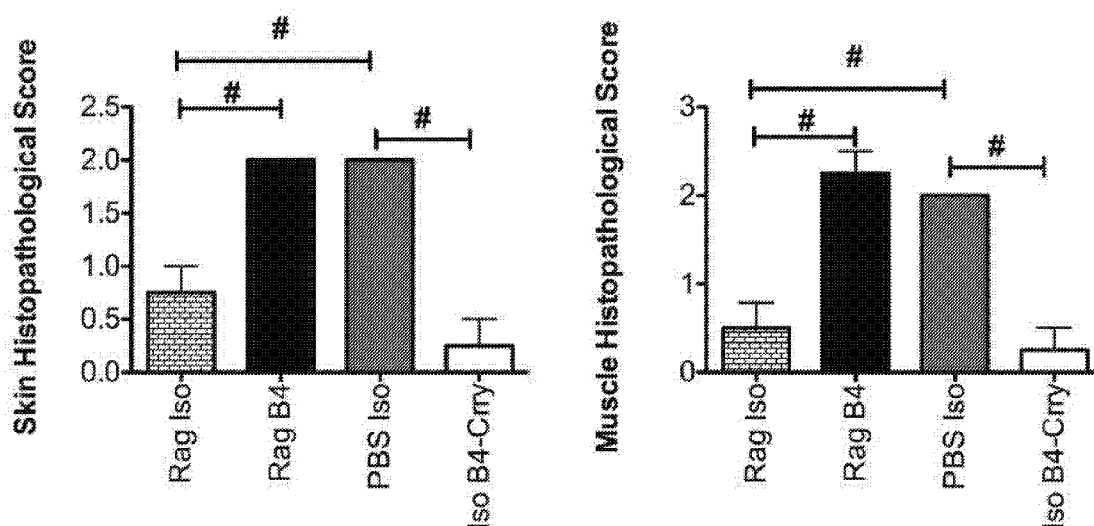
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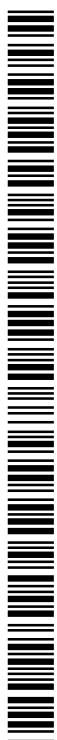
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Figure 1



(57) Abstract: Described herein are compositions and methods for treating or preventing an injury associated with transplant. Specifically, the disclosure provides a composition for treating or preventing an injury associated with transplantation, comprising (a) a targeted inhibitor molecule wherein the targeted inhibitor molecule comprises a targeting portion and an inhibitor portion, wherein the molecule inhibits complement pathways, and (b) a sub-therapeutic dose of an immunosuppressant agent. Further provided are methods of treating or preventing an injury associated with transplantation with activated complement by using the compositions.



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5 STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
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Successful organ or tissue transplantation (Tx) usually requires lifelong immunosuppression, and toxicity of immunosuppressive drugs is a serious concern since they can cause organ damage, metabolic dysfunction, cancer, and an increase in susceptibility to infection. An important goal of Tx research is the development of strategies to minimize immunosuppression. One approach being investigated to achieve this is the targeted delivery of immunosuppressive drugs to allografts, which by increasing drug bioavailability permits lower dosing and a reduction in systemic levels of immunosuppression.

25 Following severe facial injury or limb loss, transplantation (Tx) is an
accepted surgical approach for face or limb replacement, and Tx of composite tissue is
required since such injuries involve multiple tissues. Reconstructive surgery involving
vascularized composite (VC) allotransplantation (VCA) is an emerging field, with about
150-200 procedures having been performed since the first successful hand transplant in
30 1998 (Dubernard et al., 1999, Lancet, 353(9161): 1315-1320).

However, due to the heterogenicity of tissues and the high immunogenicity of skin, tissue transplantation, such as VCA, generates a strong immunological response and requires aggressive, and life-long immunosuppression. An increased concern for this type of Tx is the toxicity of immunosuppressive drugs that can cause organ damage, metabolic dysfunction, cancer, and an increase in susceptibility to infection. These toxicities are applicable for all transplant patients, and an important goal in all Tx research, is the development of strategies to minimize immunosuppression.

While graft rejection is principally dependent on T cells, there are other immune factors that can increase graft antigenicity leading to a strengthening of the rejection response. Of these other immune factors, ischemia reperfusion injury (IRI) and brain death (BD) induced injury (BDI) are thought to be the most significant risk factors for subsequent organ dysfunction and rejection. BDI is associated with increased IRI and alloresponsiveness, and is thought to be a major impediment to tolerance induction. There is currently no approved therapeutic for the treatment of IRI.

However, insight into the influence of IRI in graft rejection has been gained in studies on solid organ Tx, and it has been proposed that composite tissue allografts are more susceptible to IRI than solid organs because of the heterogeneous tissue types that all exhibit different immunogenicity (Caterson et al., 2013, The Journal of craniofacial surgery, 24(1): 51-56). Little is known about how IRI modulates longer-term outcomes after VCA, but increased ischemia times are associated with more frequent and more severe acute rejections with increased anti-donor lymphocyte proliferation following VCA (Xiao et al., 2010, The Journal of surgical research, 164(2): e299-e304, Pradka et al., 2009, Transplantation proceedings, 41(2): 531-536, Shimizu et al., 2010, Microsurgery. 2010;30(2): 132-137). In the case of VCA, although acute rejection episodes can usually be successfully controlled, the number and severity of these episodes are associated with chronic rejection.

In the context of heart Tx, primary graft failure and cardiac allograft vasculopathy (AV) remain the major limitations to short and long-term survival. Indeed, the course, severity and onset of AV have changed little since the inception of cardiac Tx surgery, despite improvements in T cell immunosuppression. The precise mechanisms

involved in the development of primary graft failure and chronic AV are not well understood, but studies indicate that BD and IRI play roles in the development of AV.

There is thus a need in the art for compositions and methods for treating and preventing injury associated with transplantation. The present invention addresses
5 this unmet need in the art.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a composition for treating or preventing an injury associated with transplantation, comprising a targeted inhibitor
10 molecule. In one embodiment, the targeted inhibitor molecule comprises a targeting portion and an inhibitor portion. In one embodiment, the molecule inhibits complement pathways. In one embodiment the composition comprises a sub-therapeutic dose of an immunosuppressant agent.

In one embodiment, the targeting portion of the molecule comprises an
15 antibody or fragment thereof. In one embodiment, the antibody or antibody portion specifically binds to Annexin IV, a post-translational modification found on Annexin IV and other proteins, or a phospholipid.

In one embodiment, the inhibitor portion of the molecule comprises at least one selected from the group consisting of FH, MCP, DAF, Crry, MAp44, CD59,
20 and CR1.

In one embodiment, the composition comprises an immunosuppressant selected from the group consisting of cyclosporine A, azathioprine, cyclophosphamide, FK506. In one embodiment, the immunosuppressant agent comprises a corticosteroid including prednisone and methylprednisolone. In one embodiment, the
25 immunosuppressant agent comprises an mTOR inhibitor selected from a group consisting of rapamycin, sirolimus, and everolimus.

In one aspect, the present invention provides a method of treating or preventing an injury associated with transplantation wherein complement is activated. In one embodiment, the method comprises administering to a subject a therapeutically
30 effective amount of a therapeutic agent comprising a targeted inhibitor molecule. In one

embodiment the targeting inhibitor molecule comprises a targeting portion and an inhibitor portion wherein the molecule inhibits complement activation.

In one embodiment, the transplant comprises a vascularized composite allograft, heart transplant, kidney transplant, liver transplant, lung transplant, pancreas
5 transplant, intestine transplant, thymus transplant, musculoskeletal graft, cornea graft, skin graft, heart valve graft, nerves graft or vein graft.

In one embodiment, the transplant comprises an allogeneic hematopoietic stem cell transplantation.

In one embodiment, the targeting portion of the composition of the method
10 comprises an antibody fragment binds to Annexin IV, a post-translational modification found on Annexin IV and other proteins, or a phospholipid.

In one embodiment, the inhibitor portion of the composition of the method comprises at least one selected from the group consisting of FH, MCP, DAF, Crry, Map44, CD59, and CR1.

15 In one embodiment, the method further comprises administering to the subject a sub-therapeutic amount of an immunosuppressant agent.

In one embodiment, the immunosuppressant agent comprises an agent selected from the group consisting of cyclosporine A, azathioprine, cyclophosphamide, FK506. In one embodiment, the immunosuppressant agent comprises a corticosteroid
20 including prednisone and methylprednisolone. In one embodiment, the immunosuppressant agent comprises an mTOR inhibitor selected from a group consisting of rapamycin, sirolimus, and everolimus.

In one embodiment, the subject has graft versus host disease.

In one embodiment, the subject has an ischemia reperfusion injury of the
25 transplant or is at risk for developing an ischemia reperfusion injury of the transplant.

In one aspect, the present invention provides a method of treating or preventing an injury associated with a transplant. In one embodiment, the method comprises administering to a transplant a therapeutically effective amount of a therapeutic agent comprising a targeted inhibitor molecule comprising a targeting portion
30 and an inhibitor portion. In one embodiment, the molecule inhibits complement pathways.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of preferred embodiments of the invention will be better understood when read in conjunction with the appended
5 drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

Figure 1 is a set of graphs demonstrating that B4-Crry treatment protects
10 against IRI of VCA grafts. Shown are: Ischemia reperfusion injury (IRI) VC isografts in PBS treated recipients (PBS Iso); protection from IRI in Rag-/- mice that do not contain Abs (Rag Iso); restoration of IRI in Rag-/- mice when reconstituted with B4 IgM mAb; and protection from IRI in wild type mice treated with B4scFv-Crry (Iso B4-Crry).

Figure 2 depicts C3d deposition at GVHD target organs.

15 Figure 3 depicts that deficiency of FB but not C1q/MLB in that host ameliorates GVHD.

Figure 4, comprising Figure 4A through Figure 4C depicts mechanisms for GVHD attenuation caused by host complement pathway deficiency.

Figure 5 depicts the Binding of ¹²⁵I-B4-Crry after perfusion of grafts of
20 BD (3 hours) or living donors. Heparin was injected into the inferior vena cava, and 0.2ml radiolabeled protein was injected into proximal ascending aorta for perfusion. After 5 minutes, flush with 0.5ml PBS, weigh and count.

Figure 6 depicts the results of example experiments depicting the survival curve from B4-Crry and subtherapeutic tacrolimus. Acute B4-Crry treatment combined
25 with subtherapeutic immunosuppression with tacrolimus significantly prolongs graft survival, as compared to control tacrolimus alone. n=5 p<0.001.

Figure 7 depicts the nucleic acid sequence and amino acid sequence for B4Crry.

Figure 8 depicts the nucleic acid sequence and amino acid sequence for
30 C2Crry.

DETAILED DESCRIPTION

The present invention is directed to compositions and methods for treating or preventing injury associated with tissue transplantation. For example, in one
5 embodiment, the invention provides for a reduced required dose of immunosuppression to prevent organ/tissue rejection or vasculopathy. For example, in certain aspects, the present invention provides compositions and methods for treatment, inhibition, prevention or reduction of the inflammatory responses associated with the complement signaling. In particular, the present invention may treat or prevent injuries such as donor
10 brain death induced injuries (BDI) and ischemia-reperfusion injuries (IRI) that occur in tissue/organ transplants such as vascular composite allografts (VCA) and injuries resulting from graft-versus-host disease (GVHD) that can develop following allogeneic hematopoietic stem cell transplantation (all-HSCT). In particular, the invention is related to compositions and methods affecting signaling associated with complement and
15 products of complement activation thereof associated with transplants particularly associated with VCA and all-HSCT.

A potential problem in the translation of a complement inhibitor strategy to the clinic is the immunosuppressive effect of systemic complement inhibition, especially important in a Tx setting where the patient is immunocompromised. Also,
20 complement has important roles in homeostatic and physiological functions such as immune complex catabolism, clearance of dead and dying cells, tissue repair, modulation of adaptive immunity, neuroregenerative processes and host defense. Other important concerns regarding the use of systemic complement inhibition relate to efficacy and biodistribution. An approach to alleviate the concerns of systemic inhibition described
25 herein specifically targets complement inhibition to sites of complement activation. In this approach, an antibody or fragment thereof that recognizes Annexin IV or a phospholipid is linked to a complement inhibitor. It is demonstrated that site-specific targeting of a complement inhibitor obviates the need for systemic inhibition and increases bioavailability and efficacy, without affecting susceptibility to infection, unlike
30 systemic complement inhibition. Targeted complement inhibition has specific value in

Tx, since much of the immune priming and activation occurs initially following BD and during the IRI period within the grafted tissues.

In certain instances, the targeting strategy described herein is advantageous for treating and preventing transplantation-induced injury. First, it will target the proximal event in complement activation, and does not depend on prior complement activation (unlike CR2). Second, the targeting vehicle itself contributes to therapeutic activity by blocking the binding of complement activating pathogenic IgM, which in turn reduces the binding of C1q and MBL, which can impact inflammation, endothelial activation, cell trafficking, and Ag-presentation. Third, it is likely less immunosuppressive since not all sites of infection and C3 deposition will be targeted with complement inhibition. Fourth, it does not limit expression of its own ligand.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

As used herein, each of the following terms has the meaning associated with it in this section.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

“About” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, or $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

As used herein, “conjugated” refers to covalent attachment of one molecule to a second molecule.

A “disease” is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal’s health continues to deteriorate.

5 The terms “effective amount” and “pharmaceutically effective amount” or “therapeutically effective amount” refer to a sufficient amount of an agent to provide the desired biological result. That result can be reduction and/or alleviation of a sign, symptom, or cause of a disease or disorder, or any other desired alteration of a biological system. An appropriate effective amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

10 The term "fusion protein" used herein refers to two or more peptides, polypeptides, or proteins operably linked to each other.

“Graft” refers to a cell, tissue, organ or otherwise any biological compatible substrate for transplantation.

15 An "individual" is a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, mice and rats. In some embodiments, the individual is human. In some embodiments, the individual is an individual other than human.

20 The term “inhibit,” as used herein, means to suppress or block an activity or function relative to a control value. Preferably, the activity is suppressed or blocked by 10% compared to a control value, more preferably by 50%, and even more preferably by 95%.

25 Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

30 As used herein, the terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein’s or peptide’s sequence. Polypeptides include any peptide or protein comprising

two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

10 The term "pharmaceutically acceptable" as used herein, refers to agents that, within the scope of sound medical judgment, are suitable for use in contact with tissues of human beings and/or animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

15 The terms "subject," "patient," "individual," and the like are used interchangeably herein, and refer to any animal, or cells thereof whether *in vitro* or *in situ*, amenable to the methods described herein. In certain non-limiting embodiments, the patient, subject or individual is a human.

 The term "sub-therapeutic" as used herein means a treatment at a dose known to be less than what is known to induce a therapeutic effect.

20 The term "therapeutic" as used herein means a treatment and/or prophylaxis. A therapeutic effect is obtained by suppression, remission, or eradication of a disease state.

 The term "therapeutic agent" use herein refers to any agent that has a therapeutic effect and/or elicits a desired biological and/or pharmacological effect, when administered to a subject. In some embodiments, an agent is considered to be a therapeutic agent if its administration to a relevant population is statistically correlated with a desired or beneficial therapeutic outcome in the population, whether or not a particular subject to whom the agent is administered experiences the desired or beneficial therapeutic outcome.

The term "therapeutically effective amount" as used herein, means an amount that is sufficient, when administered to a population suffering from or susceptible to a disease, disorder, and/or condition in accordance with a therapeutic dosing regimen, to treat the disease, disorder, and/or condition (e.g., host versus graft disease). In some
5 embodiments, a therapeutically effective amount is one that reduces the incidence and/or severity of, and/or delays onset of, one or more symptoms of the disease, disorder, and/or condition. Those of ordinary skill in the art will appreciate that the term "therapeutically effective amount" does not in fact require successful treatment be achieved in a particular individual. Rather, a therapeutically effective amount may be that amount that provides a
10 particular desired pharmacological response in a significant number of subjects when administered to patients in need of such treatment. It is specifically understood that particular subjects may, in fact, be "refractory" to a "therapeutically effective amount." To give but one example, a refractory subject may have a low bioavailability such that clinical efficacy is not obtainable. In some embodiments, reference to a therapeutically
15 effective amount may be a reference to an amount as measured in one or more specific tissues (e.g., a tissue affected by the disease, disorder or condition) or fluids (e.g., blood, saliva, serum, sweat, tears, urine, etc.). Those of ordinary skill in the art will appreciate that, in some embodiments, a therapeutically effective agent may be formulated and/or administered in a single dose. In some embodiments, a therapeutically effective agent
20 may be formulated and/or administered in a plurality of doses, for example, as part of a dosing regimen.

The term "tissue transplantation", as used herein, refers to a transfer of tissue or tissue components from an external source into a recipient (host) individual. In some embodiments, the external source is an organism (e.g., a living, brain dead, recently
25 dead, or dead organism). In some embodiments, the external source is an ex vivo or in vitro system.

A "vector" is a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear
30 polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "vector" includes an autonomously replicating

plasmid or a virus. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors,
5 lentiviral vectors, and the like.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be
10 considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies
15 regardless of the breadth of the range.

Description

This invention describes a therapeutic composition and method for treating or preventing injury associated with transplantation. For example, in certain
20 embodiments, the invention reduces the required dose of immunosuppression to prevent organ/tissue rejection or vasculopathy. In one embodiment, the composition comprises a targeted complement inhibitor and an immunosuppressant agent. In certain embodiments, the complement inhibitor is a composite molecule comprised of a targeting portion and an inhibitor portion wherein the composite molecule targets complement signaling. In
25 certain embodiments, the targeting portion comprises an antibody or a fragment thereof that specifically binds to Annexin IV, a post-translational modification found on Annexin IV and other proteins, or a phospholipid.

In one embodiment, the composition comprises a targeted complement inhibitor and an immunosuppressant agent. In one embodiment, the composition
30 comprises a sub-therapeutic level of the immunosuppressant agent. For example, in certain embodiments, the sub-therapeutic level of the immunosuppressant agent is a level

of the immunosuppressant agent that has little to no efficacy if used alone. In certain embodiments, the immunosuppressant agent comprises cyclosporine A. In certain instances, the present invention significantly prolongs graft viability without the need for traditional anti-rejection therapies including but not limited to therapeutic levels of immunosuppression therapy.

The present invention is based in part on the discovery that targeted complement inhibitor molecules efficaciously reduce activation of the complement pathways reducing inflammation in many indications including atherosclerosis, multiple sclerosis and IRI. Accordingly, the present invention relates to compositions and methods for improving the viability of vascular composite allografts (VCA), reducing transplant damage, and reducing the risk to the recipient relating to the side effects around graft rejection including those involving the complement pathway.

In one aspect, the present invention relates to a composition used to treat a subject that is a recipient of a transplant. In one embodiment, the composition modulates complement signaling. In certain instances, the composition of the present invention comprises a composite molecule comprising a targeting portion and an inhibitor portion. In particular, the targeting portion directs the molecule to sites of injury or inflammation, and the inhibitor portion inhibit complement signaling. In some instances, the targeting portion comprises an antibody or a fragment thereof that specifically binds to Annexin IV, a post-translational modification found on Annexin IV and other proteins, or a phospholipid. In certain embodiments, the targeting portion comprises B4 or C2, or fragments thereof. In some instances, the inhibitor portion is selected from a list comprising but not limited to Factor H (FH), Crry, DAF, MCP, MAp44, and CR1. In certain instances, the composition is the combination of the composite molecule in addition to a sub-therapeutic amount of an immunosuppressant agent. In some instances, the immunosuppressant therapeutic agent selected from a list of agents comprising cyclosporine A azathioprine, corticosteroids including prednisone, and methylprednisolone, cyclophosphamide, FK506, and mTOR inhibitors including rapamycin, sirolimus, and everolimus.

Targeted Complement Inhibitor

In some embodiments, there is provided a molecule (or a composition comprising the molecule such as a pharmaceutical composition), wherein the molecule comprises (a) an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV, a post-translational modification found on
5 Annexin IV and other proteins, or a phospholipid; and (b) an inhibitor portion.

In some embodiments, the antibody or a fragment thereof specifically binds to a post-translational modification, including, but not limited to, glycosylation, phosphorylation, acetylation, methylation, myristoylation, prenylation, palmitation, amidation, and lipidation, of one or more residues of a protein.

10 In some embodiments, the molecule comprises an inhibitor portion (such as a complement modulator, for example a complement inhibitor). In some embodiments, the molecule is a fusion protein. In some embodiments, the antibody or fragment thereof (hereinafter also referred to as the "targeting portion" and the inhibitor portion are linked via a linker (such as a peptide linker). In some embodiments, the targeting portion and
15 inhibitor portion are directly linked.

In some embodiments, the targeting portion comprises an antibody or fragment thereof, wherein the antibody or fragment thereof comprises: (i) a light chain variable domain comprising a sequence of SEQ ID NO: 1, a sequence of SEQ ID NO:2, or a sequence of SEQ ID NO:3; and/or (ii) heavy chain variable domain comprising a
20 sequence of SEQ ID NO:4, a sequence of SEQ ID NO:5, or a sequence of SEQ ID NO:6.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins; wherein the antibody or fragment thereof comprises: (i) a light chain variable domain
25 comprising a sequence of SEQ ID NO:7, a sequence of SEQ ID NO:8, or a sequence of SEQ ID NO:9; and/or (ii) heavy chain variable domain comprising a sequence of SEQ ID NO: 10, a sequence of SEQ ID NO: 11, or a sequence of SEQ ID NO: 12. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody B4) to Annexin IV. In some
30 embodiments, the antibody or fragment thereof binds to the same epitope as a pathogenic antibody (such as a monoclonal antibody B4) to Annexin IV. In some embodiments, the

Annexin IV is present on the surface of a cell (and/or in a pathological structure) or in the extracellular matrix or in the extracellular matrix in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the Annexin IV is produced by a nucleated cell (such as a mammalian cell). In some embodiments, the Annexin IV is recombinant protein.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins; wherein the antibody or fragment thereof comprises: (i) a light chain variable domain comprising a sequence of SEQ ID NO: 1; (ii) a light chain variable domain comprising a sequence of SEQ ID NO:2; and (iii) a light chain variable domain comprising a sequence of SEQ ID NO:3. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins; wherein the antibody or fragment thereof comprises: (i) a light chain variable domain comprising a sequence of SEQ ID NO:7; (ii) a light chain variable domain comprising a sequence of SEQ ID NO: 8; and (iii) a light chain variable domain comprising a sequence of SEQ ID NO:9. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody B4) to Annexin IV. In some embodiments, the antibody or fragment thereof binds to the same epitope as a pathogenic antibody (such as a monoclonal antibody B4) to Annexin IV. In some embodiments, the Annexin IV is present on the surface of a cell (and/or in a pathological structure) or in the extracellular matrix in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the Annexin IV is produced by a nucleated cell (such as a mammalian cell). In some embodiments, the Annexin IV is recombinant protein.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins; wherein the antibody or fragment thereof comprises: (i) heavy chain variable domain comprising a sequence of SEQ ID NO:4; (ii) heavy chain variable domain comprising a

sequence of SEQ ID NO:5; and (iii) heavy chain variable domain comprising a sequence of SEQ ID NO:6. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins, wherein the antibody or fragment thereof comprises: (i) heavy chain variable domain comprising a sequence of SEQ ID NO: 10; (ii) heavy chain variable domain comprising a sequence of SEQ ID NO: 11; and (iii) heavy chain variable domain comprising a sequence of SEQ ID NO: 12. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody B4) to Annexin IV. In some embodiments, the antibody or fragment thereof binds to the same epitope as a pathogenic antibody (such as a monoclonal antibody B4) to Annexin IV. In some embodiments, the Annexin IV is present on the surface of a cell (and/or in a pathological structure) or in the extracellular matrix in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the Annexin IV is produced by a nucleated cell (such as a mammalian cell). In some embodiments, the Annexin IV is recombinant protein.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins; wherein the antibody or fragment thereof comprises: (i) a light chain variable domain comprising a sequence of SEQ ID NO: 1; (ii) a light chain variable domain comprising a sequence of SEQ ID NO:2; (iii) a light chain variable domain comprising a sequence of SEQ ID NO:3; (iv) heavy chain variable domain comprising a sequence of SEQ ID NO:4; (v) heavy chain variable domain comprising a sequence of SEQ ID NO:5; and (vi) heavy chain variable domain comprising a sequence of SEQ ID NO:6. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins, wherein the antibody or fragment thereof comprises: (i) a light chain variable domain comprising a sequence of SEQ ID NO:7; (ii) a light chain variable domain comprising a sequence of SEQ ID NO: 8; (iii) a light chain variable domain comprising a sequence of SEQ ID NO:9; (iv) heavy chain

variable domain comprising a sequence of SEQ ID NO: 10; (v) heavy chain variable domain comprising a sequence of SEQ ID NO: 11; and (vi) heavy chain variable domain comprising a sequence of SEQ ID NO: 12. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody B4) to Annexin IV. In some embodiments, the antibody or fragment thereof binds to the same epitope as a pathogenic antibody (such as a monoclonal antibody B4) to Annexin IV. In some embodiments, the Annexin IV is present on the surface of a cell (and/or in a pathological structure) or in the extracellular matrix in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the Annexin IV is produced by a nucleated cell (such as a mammalian cell). In some embodiments, the Annexin IV is recombinant protein.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins, wherein the antibody or fragment thereof comprises: (i) a light chain CDR1 of SEQ ID NO: 1; (ii) a light chain CDR2 of SEQ ID NO:2; (iii) a light chain CDR3 of SEQ ID NO:3; (iv) heavy chain CDR1 of SEQ ID NO:4; (v) heavy chain CDR2 of SEQ ID NO:5; and (vi) heavy chain CDR3 of SEQ ID NO:6. In some embodiments the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins; and (b) a complement modulator or a detectable moiety, wherein the antibody or fragment thereof comprises: (i) a light chain CDR1 of SEQ ID NO:7; (ii) a light chain CDR2 of SEQ ID NO: 8; (iii) a light chain CDR3 of SEQ ID NO:9; (iv) heavy chain CDR1 of SEQ ID NO: 10; (v) heavy chain CDR2 of SEQ ID NO: 11 ; and (vi) heavy chain CDR3 of SEQ ID NO: 12. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody B4) to Annexin IV. In some embodiments, the antibody or fragment thereof binds to the same epitope as a pathogenic antibody (such as a monoclonal antibody B4) to Annexin IV. In some embodiments, the Annexin IV is present on the surface of a cell (and/or in a pathological structure) or in the extracellular matrix in an

individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the Annexin IV is produced by a nucleated cell (such as a mammalian cell). In some embodiments, the Annexin IV is recombinant protein.

5 In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins, wherein the antibody or fragment thereof comprises a light chain variable domain of SEQ ID NO: 13. In some embodiments, the targeting portion comprises an antibody or a
10 fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins, wherein the antibody or fragment thereof comprises a heavy chain variable domain of SEQ ID NO: 15. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to
15 Annexin IV or a post-translational modification found on Annexin IV and other proteins, wherein the antibody or fragment thereof comprises a light chain variable domain of SEQ ID NO: 14. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins,
20 wherein the antibody or fragment thereof comprises a heavy chain variable domain of SEQ ID NO: 16. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic monoclonal antibody (such as monoclonal antibody B4) to Annexin IV. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody
25 B4) to Annexin IV. In some embodiments, the antibody or fragment thereof binds to the same epitope as a pathogenic antibody (such as a monoclonal antibody B4) to Annexin IV. In some embodiments, the Annexin IV is present on the surface of a cell (and/or in a pathological structure) or in the extracellular matrix in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage.
30 In some embodiments, the Annexin IV is produced by a nucleated cell (such as a mammalian cell). In some embodiments, the Annexin IV is recombinant protein

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins, wherein the antibody or fragment thereof comprises: (i) a light chain variable domain of SEQ ID NO: 13; and (ii) heavy chain variable domain of SEQ ID NO: 15. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins, wherein the antibody or fragment thereof comprises: (i) a light chain variable domain of SEQ ID NO: 14; and (ii) heavy chain variable domain of SEQ ID NO: 16. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody B4) to Annexin IV. In some embodiments, the antibody or fragment thereof binds to the same epitope as a pathogenic antibody (such as a monoclonal antibody B4) to Annexin IV. In some embodiments, the Annexin IV is present on the surface of a cell (and/or in a pathological structure) or in the extracellular matrix in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the Annexin IV is produced by a nucleated cell (such as a mammalian cell). In some embodiments, the Annexin IV is recombinant protein.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins, wherein the antibody or fragment is a scFv having the sequence of SEQ ID NO: 17. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a post-translational modification found on Annexin IV and other proteins, wherein the antibody or fragment is a scFv having the sequence of SEQ ID NO: 18. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody B4) to Annexin IV. In some embodiments, the antibody or fragment thereof binds to the same epitope as a pathogenic antibody (such as a monoclonal antibody B4) to Annexin IV. In some embodiments, the Annexin IV is

present on the surface of a cell (and/or in a pathological structure) or in the extracellular matrix in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the Annexin IV is produced by a nucleated cell (such as a mammalian cell). In some embodiments, the
5 Annexin IV is recombinant protein.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as phosphatidylethanolamine (PE), cardiolipin (CL), malondialdehyde (MDA) and/or phosphatidylcholine (PC)), wherein the antibody or
10 fragment thereof comprises: (i) a light chain variable domain comprising a sequence of SEQ ID NO:25, a sequence of SEQ ID NO:26, or a sequence of SEQ ID NO:27; and/or (ii) heavy chain variable domain comprising a sequence of SEQ ID NO:28, a sequence of SEQ ID NO:29, or a sequence of SEQ ID NO:30. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment
15 thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises: (i) a light chain variable domain comprising a sequence of SEQ ID NO:31, a sequence of SEQ ID NO:32, or a sequence of SEQ ID NO:33; and/or (ii) heavy chain variable domain comprising a sequence of SEQ ID NO:28, a sequence of SEQ ID NO:29, or a sequence of SEQ ID NO:30. In some
20 embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the antibody or antibody fragment thereof binds to the same epitope as a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some
25 embodiments, the phospholipid is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the phospholipid is selected from the group consisting of phosphatidylethanolamine (PE), cardiolipin (CL), and phosphatidylcholine (PC). In some embodiments, the phospholipid is malondialdehyde (MDA). In some embodiments, the phospholipid is neutral. In some
30 embodiments, the phospholipid is positively charged. In some embodiments, the phospholipid is oxidized.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises: (i) a light chain variable domain comprising a sequence of SEQ ID NO:25; (ii) a light chain variable domain comprising a sequence of SEQ ID NO:26; and (iii) a light chain variable domain comprising a sequence of SEQ ID NO:27. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises: (i) a light chain variable domain comprising a sequence of SEQ ID NO:31; (ii) a light chain variable domain comprising a sequence of SEQ ID NO:32; and (iii) a light chain variable domain comprising a sequence of SEQ ID NO:33. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the antibody or antibody fragment thereof binds to the same epitope as a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the phospholipid is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the phospholipid is selected from the group consisting of phosphatidylethanolamine (PE), cardiolipin (CL), and phosphatidylcholine (PC). In some embodiments, the phospholipid is malondialdehyde (MDA). In some embodiments, the phospholipid is neutral. In some embodiments, the phospholipid is positively charged. In some embodiments, the phospholipid is oxidized.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises: (i) heavy chain variable domain comprising a sequence of SEQ ID NO:28; (ii) heavy chain variable domain comprising a sequence of SEQ ID NO:29; and (iii) heavy chain variable domain comprising a sequence of SEQ ID NO:30. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some

embodiments, the antibody or antibody fragment thereof binds to the same epitope as a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the phospholipid is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to a tissue undergoing
5 (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the phospholipid is selected from the group consisting of phosphatidylethanolamine (PE), cardiolipin (CL), and phosphatidylcholine (PC). In some embodiments, the phospholipid is malondialdehyde (MDA). In some embodiments, the phospholipid is neutral. In some
10 embodiments, the phospholipid is positively charged. In some embodiments, the phospholipid is oxidized.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises: (i) a light chain variable domain comprising a sequence of SEQ ID
15 NO:25; (ii) a light chain variable domain comprising a sequence of SEQ ID NO:26; (iii) a light chain variable domain comprising a sequence of SEQ ID NO:27; (iv) heavy chain variable domain comprising a sequence of SEQ ID NO:28; (v) heavy chain variable domain comprising a sequence of SEQ ID NO:29; and (vi) heavy chain variable domain comprising a sequence of SEQ ID NO:30. In some embodiments, the targeting portion
20 comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises: (i) a light chain variable domain comprising a sequence of SEQ ID NO:31; (ii) a light chain variable domain comprising a sequence of SEQ ID NO:32; (iii) a light chain variable domain comprising a sequence of SEQ ID
25 NO:33; (iv) heavy chain variable domain comprising a sequence of SEQ ID NO:28; (v) heavy chain variable domain comprising a sequence of SEQ ID NO:29; and (vi) heavy chain variable domain comprising a sequence of SEQ ID NO:30. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the
30 antibody or antibody fragment thereof binds to the same epitope as a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the

phospholipid is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the phospholipid is selected from the group consisting of phosphatidylethanolamine (PE),
5 cardiolipin (CL), and phosphatidylcholine (PC). In some embodiments, the phospholipid is malondialdehyde (MDA). In some embodiments, the phospholipid is neutral. In some embodiments, the phospholipid is positively charged. In some embodiments, the phospholipid is oxidized.

In some embodiments, the targeting portion comprises an antibody or a
10 fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises: (i) a light chain CDR1 of SEQ ID NO:25; (ii) a light chain CDR2 of SEQ ID NO:26; (iii) a light chain CDR3 of SEQ ID NO:27; (iv) heavy chain CDR1 of SEQ ID NO:28; (v) heavy chain CDR2 of SEQ ID NO:29; and (vi) heavy chain CDR3 of
15 SEQ ID NO:30. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises: (i) a light chain CDR1 of SEQ ID NO:31; (ii) a light chain CDR2 of SEQ ID NO:32; (iii) a light chain CDR3 of SEQ ID NO:33; (iv) heavy chain CDR1 of
20 SEQ ID NO:28; (v) heavy chain CDR2 of SEQ ID NO:29; and (vi) heavy chain CDR3 of SEQ ID NO:30. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the antibody or antibody fragment thereof binds to the same epitope as a pathogenic antibody (such as monoclonal antibody C2) to
25 phospholipid. In some embodiments, the phospholipid is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the phospholipid is selected from the group consisting of phosphatidylethanolamine (PE), cardiolipin (CL), and phosphatidylcholine (PC). In some
30 embodiments, the phospholipid is malondialdehyde (MDA). In some embodiments, the

phospholipid is neutral. In some embodiments, the phospholipid is positively charged. In some embodiments, the phospholipid is oxidized.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises a light chain variable domain of SEQ ID NO:34. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises a heavy chain variable domain of SEQ ID NO:36. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises a light chain variable domain of SEQ ID NO:35. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the antibody or antibody fragment thereof binds to the same epitope as a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the phospholipid is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the phospholipid is selected from the group consisting of phosphatidylethanolamine (PE), cardiolipin (CL), and phosphatidylcholine (PC). In some embodiments, the phospholipid is malondialdehyde (MDA). In some embodiments, the phospholipid is neutral. In some embodiments, the phospholipid is positively charged. In some embodiments, the phospholipid is oxidized.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises: (i) a light chain variable domain of SEQ ID NO:34; and (ii) heavy chain variable domain of SEQ ID NO:36. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof

specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment thereof comprises: (i) a light chain variable domain of SEQ ID NO:35; and (ii) heavy chain variable domain of SEQ ID NO:36. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the antibody or antibody fragment thereof binds to the same epitope as a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the phospholipid is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the phospholipid is selected from the group consisting of phosphatidylethanolamine (PE), cardiolipin (CL), and phosphatidylcholine (PC). In some embodiments, the phospholipid is malondialdehyde (MDA). In some embodiments, the phospholipid is neutral. In some embodiments, the phospholipid is positively charged. In some embodiments, the phospholipid is oxidized.

In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC); and (b) an active moiety (e.g., a therapeutic moiety or a detectable moiety), wherein the antibody or fragment is a scFv having the sequence of SEQ ID NO:37. In some embodiments, the targeting portion comprises an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to a phospholipid (such as PE, CL, MDA, and/or PC), wherein the antibody or fragment is a scFv having the sequence of SEQ ID NO:38. In some embodiments, the antibody or fragment thereof competitively inhibits the binding of a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the antibody or antibody fragment thereof binds to the same epitope as a pathogenic antibody (such as monoclonal antibody C2) to phospholipid. In some embodiments, the phospholipid is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury and/or oxidative damage. In some embodiments, the phospholipid is selected from the group consisting of phosphatidylethanolamine (PE),

cardiolipin (CL), and phosphatidylcholine (PC). In some embodiments, the phospholipid is malondialdehyde (MDA). In some embodiments, the phospholipid is neutral. In some embodiments, the phospholipid is positively charged. In some embodiments, the phospholipid is oxidized.

5 In some embodiments, the targeting portion and inhibitor portion are directly bonded, covalently bonded, or, reversibly bonded. The targeting portion is capable of specifically binding to Annexin IV or a phospholipid. The targeting portion is responsible for targeted delivery of the molecule to the sites of, e.g., complement activation. The inhibitor portion is responsible for therapeutic activity, e.g., specifically
10 inhibiting complement activation. The targeting portion and inhibitor portion of the molecule can be linked together by any methods known in the art, as long as the desired functionalities of the two portions are maintained.

 The molecule described herein thus generally has the dual functions of binding to an epitope recognized by an antibody described herein and exerting
15 therapeutic activity. A "epitope of monoclonal antibody B4 antibody" refers to any molecule that binds to a naturally occurring B4 or C2 antibody, which include, epitopes that bind to a B4 or C2 antibody with a binding affinity that is about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the epitope that naturally binds a B4 antibody. Binding affinity can be determined by any method known in the art, including
20 for example, surface plasmon resonance, calorimetry titration, ELISA, and flow cytometry.

 In some embodiments, a molecule described herein is generally capable of inhibiting complement activation (for example inhibiting activation of the alternative pathway, classical pathway and/or lectin pathway). The molecule may be a more potent
25 complement inhibitor than the naturally occurring antibody as described herein. For example, in some embodiments, the molecule has a complement inhibitory activity that is about any of 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, 30, 40, or more fold of that of a B4 or C2 antibody. In some embodiments, the molecule has an EC₅₀ of less than about any of 100 nM, 90 nM, 80 nM, 70 nM, 60 nM, 50 nM, 40 nM, 30 nM, 20
30 nM, or 10 nM, inclusive, including any values in between these numbers. In some embodiments, the molecule has an EC₅₀ of about 5 to 60 nM, including for example any

of 8 to 50 nM, 8 to 20 nM, 10 to 40 nM, and 20 to 30 nM. In some embodiments, the molecule has complement inhibitory activity that is about any of 50%, 60%, 70%, 80%, 90%, or 100% of that of a B4 or C2 antibody.

Complement inhibition can be evaluated based on any methods known in the art, including for example, in vitro zymosan assays, assays for lysis of erythrocytes, antibody or immune complex activation assays, alternative pathway activation assays, classical pathway activation assays and mannan (lectin pathway) activation assays.

In some embodiments, the molecule is a fusion protein. "Fusion protein" used herein refers to two or more peptides, polypeptides, or proteins operably linked to each other. In some embodiments, the targeting portion and inhibitor portion are directly fused to each other. In some embodiments, the targeting portion and inhibitor portion are linked by an amino acid linker sequence. Examples of linker sequences are known in the art, and include, for example, (Gly4Ser), (Gly4Ser)₂, (Gly4Ser)₃, (Gly3Ser)₄, (SerGly4), (SerGly4)₂, (SerGly4)₃, and (SerGly4)₄. Linking sequences can also comprise "natural" linking sequences found between different domains of complement factors. The order of targeting portion and inhibitor portion in the fusion protein can vary. For example, in some embodiments, the C-terminus of the targeting portion is fused (directly or indirectly) to the N-terminus of the inhibitor portion of the targeting construct. In some embodiments, the N-terminus of the targeting portion is fused (directly or indirectly) to the C-terminus of the inhibitor portion of the molecule. In some embodiments, the targeting portion is encoded by a polynucleotide comprising a nucleic acid sequence of any of 19-24, 39-43, 57 and 58. In some embodiments, the targeting portion is encoded by a polynucleotide comprising a nucleic acid sequence that is at least about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to that of any of SEQ ID NOs: 19-24, 39-43, 57 and 58.

In some embodiments, the molecule comprises a targeting portion and an inhibitor portion linked via a chemical cross-linker. Linking of the two portions can occur on reactive groups located on the two moieties. Reactive groups that can be targeted using a crosslinker include primary amines, sulfhydryls, carbonyls, carbohydrates, and carboxylic acids, or active groups that can be added to proteins. Examples of chemical linkers are well known in the art and include, but are not limited to, bismaleimido-hexane,

maleimidobenzoyl-N-hydroxysuccinimide ester, NHS-Esters-Maleimide Crosslinkers such as SPDP, carbodiimide, glutaraldehyde, MBS, Sulfo-MBS, SMPB, sulfo-SMPB, GMBS, Sulfo-GMBS, EMCS, Sulfo-EMCS, imidoester crosslinkers such as DMA, DMP, DMS, DTBP, EDC and DTME.

5 In some embodiments, the targeting portion and inhibitor portion are non-covalently linked. For example, the two portions may be brought together by two interacting bridging proteins (such as biotin and streptavidin), each linked to a targeting portion or an inhibitor portion

10 In some embodiments, the targeting portion comprises two or more (same or different) targeting portions described herein. In some embodiments, the molecule comprises two or more (same or different) inhibitor portions described herein. These two or more portions may be tandemly linked (such as fused) to each other. In some embodiments, the molecule comprises a targeting portion and two or more (such as three, four, five, or more) inhibitor portions. In some embodiments, the molecule comprises an
15 inhibitor portion and two or more (such as three, four, five, or more) targeting portions. In some embodiments, the molecule comprises two or more targeting portions and two or more inhibitor portions.

 In some embodiments, there is provided an isolated targeted molecules. In some embodiments, the targeting molecules form dimers or multimers.

20 The active portion and the targeting portion in the targeted molecule can be from the same species (such as human or mouse), or from different species.

 Annexin IV belongs to a family of proteins that are Ca^{2+} and phospholipid binding proteins. The structure of annexins consists of a conserved Ca^{2+} and membrane binding core of four annexin repeats (eight for annexin IV) and variable
25 N-terminal regions. Annexins are soluble cytosolic proteins, but despite the lack of obvious signal sequences and the apparent inability to enter the classical secretory pathway, annexins have been identified in extracellular fluids or associated with the external cell surface through poorly understood binding sites. Annexin IV is predominantly produced by epithelial cells and is also found at high levels in lung,
30 intestine, pancreas, liver, photoreceptors, and kidney. Rescher et al., J. Cell Sci., (2004),

117:2631-2639, Kulik et al., (2009) J Immunol. 182(9):5363-73, and Zernii et al., Biochemistry (Mosc)., (2003), 68(1): 129-60.

In some embodiments, the Annexin IV is present on the surface of a cell (and/or in a pathological structure) or in the extracellular matrix in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury. In some
5 embodiments, the Annexin IV is present on the surface of a cell of an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) non-ischemic injury. In some embodiments, the Annexin IV is present on the surface of a cell of an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) oxidative
10 damage. In some embodiments, the Annexin IV is present on the surface of a cell of an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) ischemia-reperfusion injury. In some embodiments, the Annexin IV is produced by a nucleated cell (such as a mammalian cell). In some embodiments, the Annexin IV is recombinant protein.

15 In some embodiments, the Annexin IV is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury. In some embodiments, the Annexin IV is present on the surface of a cell, a basement membrane (, or in a pathological structure of an individual that is in or adjacent to a tissue undergoing (or is at
20 risk of undergoing) non-ischemic injury. In some embodiments, the Annexin IV is present on the surface of a cell, a basement membrane or in a pathological structure of an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) oxidative damage. In some embodiments, the Annexin IV is present on the surface of a cell, a basement membrane, or in a pathological structure of an individual that is in or
25 adjacent to a tissue undergoing (or is at risk of undergoing) ischemia-reperfusion injury. In some embodiments, the Annexin IV is produced by a nucleated cell (such as a mammalian cell). In some embodiments, the Annexin IV is recombinant protein.

In some embodiments, the epitope on Annexin IV for the antibody or fragment thereof is present on the surface of a cell (and/or in a pathological structure) or
30 in the extracellular matrix in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury but not on the surface of a cell that is in or adjacent

to a tissue not undergoing (or is not at risk of undergoing) tissue injury. In some embodiments, the epitope on Annexin IV for the antibody or fragment thereof is present on the surface of a cell (and/or in a pathological structure) or in the extracellular matrix in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) non-ischemic injury but not on the surface of a cell that is in or adjacent to a tissue not undergoing (or is not at risk of undergoing) non-ischemic injury. In some embodiments, the epitope on Annexin IV for the antibody or fragment thereof is present on the surface of a cell that is in or adjacent to a tissue undergoing (or is at risk of undergoing) oxidative damage but not on the surface of a cell that is in or adjacent to a tissue not undergoing (or is not at risk of undergoing) oxidative damage. In some embodiments, the epitope on Annexin IV for the antibody or fragment thereof is present on the surface of a cell (and/or in a pathological structure) or in the extracellular matrix in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) ischemia-reperfusion injury but is not present on the surface of a cell that is in or adjacent to a tissue not undergoing (or is not at risk of undergoing) ischemia reperfusion injury.

In some embodiments, the epitope on Annexin IV for the antibody or fragment thereof is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury but not on the surface of a cell, a basement membrane, or in a pathological structure that is in or adjacent to a tissue not undergoing (or is not at risk of undergoing) tissue injury. In some embodiments, the epitope on Annexin IV for the antibody or fragment thereof is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) non-ischemic injury but not on the surface of a cell, a basement membrane, or in a pathological structure that is in or adjacent to a tissue not undergoing (or is not at risk of undergoing) nonischemic injury. In some embodiments, the epitope on Annexin IV for the antibody or fragment thereof is present on the surface of a cell, a basement membrane, or in a pathological structure that is in or adjacent to a tissue undergoing (or is at risk of undergoing) oxidative damage but not on the surface of a cell, a basement membrane, or in a pathological structure that is in or adjacent to a tissue not undergoing (or is not at risk of undergoing) oxidative damage. In

some embodiments, the epitope on Annexin IV for the antibody or fragment thereof is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) ischemia-reperfusion injury but is not present on the surface of a cell, a basement
5 membrane, or in a pathological structure that is in or adjacent to a tissue not undergoing (or is not at risk of undergoing) ischemia reperfusion injury.

In some embodiments, the antibody or fragment thereof described herein specifically binds to a phospholipid, which include, but is not limited to, phosphatidylethanolamine (PE), cardiolipin (CL), phosphatidylcholine (PC), and
10 malondialdehyde (MDA). PE, CL, and PC are classes of phospholipids found in biological membranes. Phosphatidylcholine is more commonly found in the exoplasmic or outer leaflet of a cell membrane. It is thought to be transported between membranes within the cell by phosphatidylcholine transfer protein (PCTP). The phospholipid is composed of a choline head group and glycerophosphoric acid with a variety of fatty
15 acids, one being a saturated fatty acid and one being an unsaturated fatty acid. PE consists of a combination of glycerol esterified with two fatty acids and phosphoric acid. Whereas the phosphate group is combined with choline in phosphatidylcholine, it is combined with the ethanolamine in PE. The two fatty acids may be the same, or different, and are usually in the 1,2 positions (though they can be in the 1,3 positions). Cardiolipin
20 (IUPAC name "1,3-bis(sn-3'-phosphatidyl)-sn-glycerol") is an important component of the inner mitochondrial membrane, where it constitutes about 20% of the total lipid composition. Cardiolipin (CL) is a kind of diphosphatidylglycerol lipid, in which two phosphatidylglycerols connect with a glycerol backbone in the center to form a dimeric structure. In most animal tissues, cardiolipin contains 18-carbon fatty alkyl chains with 2
25 unsaturated bonds on each of them. It has been proposed that the (18:2)₄ acyl chain configuration is an important structural requirement for the high affinity of CL to inner membrane proteins in mammalian mitochondria.

Malondialdehyde (MDA) is generated from reactive oxygen species (ROS), and as such is often assayed in vivo as a bio-marker of oxidative stress. Reactive
30 oxygen species degrade polyunsaturated lipids, forming malondialdehyde. This compound is a reactive aldehyde and is one of the many reactive electrophile species that

cause toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation end-products (ALE). The production of this aldehyde is also used as a biomarker to measure the level of oxidative stress in an organism.

5 In some embodiments, the phospholipid (such as PE, CL, MDA, and/or PC) is present on the surface of a cell (or in a pathological structure) in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury. In some embodiments, the phospholipid (such as PE, CL, MDA, and/or PC) is present on the surface of a cell (or in a pathological structure) of an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) ocular disease. In some embodiments, the phospholipid (such as PE, CL, MDA, and/or PC) is present on the surface of a cell (or in a pathological structure) of an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) oxidative damage. In some embodiments, the phospholipid is neutral. In some embodiments, the phospholipid is positively charged. In some embodiments, the phospholipid (such as PE, CL, MDA, and/or PC) is oxidized.

15 In some embodiments, the phospholipid (such as PE, CL, MDA, and/or PC) is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual that is in or adjacent to an ocular tissue undergoing (or is at risk of undergoing) tissue injury. In some embodiments, the phospholipid (such as PE, CL, MDA, and/or PC) is present on the surface of a cell, a basement membrane, or in a pathological structure of an individual that is in or adjacent to an ocular tissue undergoing (or is at risk of undergoing) ocular disease. In some embodiments, the phospholipid (such as PE, CL, MDA, and/or PC) is present on the surface of a cell, a basement membrane, or in a pathological structure of an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) oxidative damage. In some embodiments, the phospholipid is neutral. In some embodiments, the phospholipid is positively charged. In some embodiments, the phospholipid (such as PE, CL, MDA, and/or PC) is oxidized.

25 In some embodiments, the epitope of phospholipid (such as PE, CL, MDA, and/or PC) to which the antibody or fragment thereof binds is present on the surface of a cell or in a pathological structure in an individual that is in or adjacent to a tissue undergoing (or is at risk of undergoing) tissue injury but not on the surface of a cell or in a pathological structure that is in or adjacent to a tissue not undergoing (or is not at

risk of undergoing) tissue injury. In some embodiments, the epitope on phospholipid (such as PE, CL, MDA, and/or PC) to which the antibody or fragment thereof binds is present on the surface of a cell or in a pathological structure that is in or adjacent to a tissue undergoing (or is at risk of undergoing) oxidative damage but not on the surface of
5 a cell or in a pathological structure that is in or adjacent to a tissue not undergoing (or is not at risk of undergoing) oxidative damage.

In some embodiments, the epitope of phospholipid (such as PE, CL, MDA, and/or PC) to which the antibody or fragment thereof binds is present on the surface of a cell, a basement membrane, or in a pathological structure in an individual
10 that is in or adjacent to a ocular tissue undergoing (or is at risk of undergoing) tissue injury but not on the surface of a cell, a basement membrane, or in a pathological structure that is in or adjacent to a ocular tissue not undergoing (or is not at risk of undergoing) tissue injury. In some embodiments, the epitope on phospholipid (such as PE, CL, MDA, and/or PC) to which the antibody or fragment thereof binds is present on
15 the surface of a cell, a basement membrane, or in a pathological structure that is in or adjacent to a tissue undergoing (or is at risk of undergoing) oxidative damage but not on the surface of a cell, a basement membrane, or in a pathological structure that is in or adjacent to a tissue not undergoing (or is not at risk of undergoing) oxidative damage.

As described herein, a cell (and/or a pathological structure) that is in or
20 adjacent to a particular tissue as described herein includes a cell (and/or a pathological structure) that is part of a tissue or organ, or adjacent to (near, directly next to, in the microenvironment of, bordering, flanking, adjoining) a tissue or organ, in which a certain event (such as non-ischemic injury or oxidative damage) is going to occur, is likely to occur, or is beginning to occur. As described herein, a cell, a basement, or in a
25 pathological structure that is in or adjacent to a particular tissue as described herein includes a cell that is part of a tissue or organ, or adjacent to (near, directly next to, in the microenvironment of, bordering, flanking, adjoining) a tissue or organ, in which a certain event (such as non-ischemic injury or oxidative damage) is going to occur, is likely to occur, or is beginning to occur. In the case of an adjacent cell, the cell is sufficiently
30 within the microenvironment of the specific tissue or organ such that conditions of oxidative damage and/or inflammation affect the adjacent cell, as well as the specific

tissue or organ. Such a cell may display signs of stress, including, but not limited to, the display of "stress proteins" (e.g., heat shock proteins and other proteins associated with a cellular stress response, including annexins) or other molecules on the cell surface (phospholipids, carbohydrate moieties), including the display of abnormal levels of proteins, modified proteins, or other molecules on the cell surface. Such a cell may be undergoing apoptosis or showing signs of apoptosis, such signs including morphological changes in the cell, chromatin condensation, changes in cellular signal transduction protein interactions, changes in intracellular calcium levels, externalization of phospholipids, cell detachment, loss of cell surface structures, etc.

As used herein, the term "selectively binds to" refers to the specific binding of one protein to another protein, to a lipid, or to a carbohydrate moiety (e.g., the binding of an antibody, a fragment thereof, or binding partner to an antigen), wherein the level of binding, as measured by any standard assay (e.g., an immunoassay), is statistically significantly higher than the background control for the assay. For example, when performing an immunoassay, controls typically include a reaction well/tube that contain antibody or antigen binding fragment alone (i.e., in the absence of antigen), wherein an amount of reactivity (e.g., non-specific binding to the well) by the antibody or antigen binding fragment thereof in the absence of the antigen is considered to be background. Binding can be measured using a variety of methods standard in the art, including, but not limited to: Western blot, immunoblot, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, microcytometry, microarray, microscopy, fluorescence activated cell sorting (FACS), and flow cytometry.

According to the present invention, an "epitope" of a given protein or peptide or other molecule is generally defined, with regard to antibodies, as a part of or site on a larger molecule to which an antibody or antigen-binding fragment thereof will bind, and against which an antibody will be produced. The term epitope can be used interchangeably with the term "antigenic determinant", "antibody binding site", or "conserved binding surface" of a given protein or antigen. More specifically, an epitope

can be defined by both the amino acid residues involved in antibody binding and also by their conformation in three-dimensional space (e.g., a conformational epitope or the conserved binding surface). An epitope can be included in peptides as small as about 4-6 amino acid residues, or can be included in larger segments of a protein, and need not be
5 comprised of contiguous amino acid residues when referring to a three dimensional structure of an epitope, particularly with regard to an antibody-binding epitope.

Antibody-binding epitopes are frequently conformational epitopes rather than a sequential epitope (i.e., linear epitope), or in other words, an epitope defined by amino acid residues arrayed in three dimensions on the surface of a protein or polypeptide to
10 which an antibody binds. As mentioned above, the conformational epitope is not comprised of a contiguous sequence of amino acid residues, but instead, the residues are perhaps widely separated in the primary protein sequence, and are brought together to form a binding surface by the way the protein folds in its native conformation in three dimensions.

15 Competition assays can be performed using standard techniques in the art (e.g., competitive ELISA or other binding assays). For example, competitive inhibitors can be detected and quantitated by their ability to inhibit the binding of an antigen to a known, labeled antibody (e.g., the rriAb B4) or to sera or another composition that is known to contain antibodies against the particular antigen (e.g., sera known to contain
20 natural antibodies against the antigen).

According to the present invention, antibodies are characterized in that they comprise immunoglobulin domains and as such, they are members of the immunoglobulin superfamily of proteins. Generally speaking, an antibody molecule comprises two types of chains. One type of chain is referred to as the heavy or H chain
25 and the other is referred to as the light or L chain. The two chains are present in an equimolar ratio, with each antibody molecule typically having two H chains and two L chains. The two H chains are linked together by disulfide bonds and each H chain is linked to an L chain by a disulfide bond. There are only two types of L chains referred to as lambda (λ) and kappa (κ) chains. In contrast, there are five major H chain classes
30 referred to as isotypes. The five classes include immunoglobulin M (IgM or μ), immunoglobulin D (IgD or δ), immunoglobulin G (IgG or γ), immunoglobulin A (IgA or

a), and immunoglobulin E (IgE or ϵ). The distinctive characteristics between such isotypes are defined by the constant domain of the immunoglobulin and are discussed in detail below. Human immunoglobulin molecules comprise nine isotypes, IgM, IgD, IgE, four subclasses of IgG including IgG1 ($\gamma 1$), IgG2 ($\gamma 2$), IgG3 ($\gamma 3$) and IgG4 ($\gamma 4$), and two
5 subclasses of IgA including IgA1 (1) and IgA2 (a2). In humans, IgG subclass 3 and IgM are the most potent complement activators (classical complement system), while IgG subclass 1 and to an even lesser extent, 2, are moderate to low activators of the classical complement system. IgG4 subclass does not activate the complement system (classical or alternative). The only human immunoglobulin isotype known to activate the alternative
10 complement system is IgA. In mice, the IgG subclasses are IgG1, IgG2a, IgG2b and IgG3. Murine IgG1 does not activate complement, while IgG2a, IgG2b and IgG3 are complement activators.

Each H or L chain of an immunoglobulin molecule comprises two regions referred to as L chain variable domains (VL domains) and L chain constant domains (CL
15 domains), and H chain variable domains (VH domains) and H chain constant domains (CH domains). A complete CH domain comprises three sub-domains (CH1, CH2, CH3) and a hinge region. Together, one H chain and one L chain can form an arm of an immunoglobulin molecule having an immunoglobulin variable region. A complete immunoglobulin molecule comprises two associated (e.g., di-sulfide linked) arms. Thus,
20 each arm of a whole immunoglobulin comprises a VH+L region, and a CH+L region. As used herein, the term "variable region" or "V region" refers to a VH+L region (also known as an Fv fragment), a VL region or a VH region. Also as used herein, the term "constant region" or "C region" refers to a CH+L region, a CL region or a CH region.

The antigen specificity of an immunoglobulin molecule is conferred by the
25 amino acid sequence of a variable, or V, region. As such, V regions of different immunoglobulin molecules can vary significantly depending upon their antigen specificity. Certain portions of a V region are more conserved than others and are referred to as framework regions (FR regions). In contrast, certain portions of a V region are highly variable and are designated hypervariable regions. When the VL and VH
30 domains pair in an immunoglobulin molecule, the hypervariable regions from each domain associate and create hypervariable loops that form the antigen binding sites

(antigen combining sites). Thus, the hypervariable loops determine the specificity of an immunoglobulin and are termed complementarity-determining regions (CDRs) because their surfaces are complementary to antigens.

Both an L chain and H chain V gene segment contain three regions of substantial amino acid sequence variability. Such regions are referred to as L chain CDR1, CDR2 and CDR3, and H chain CDR1, CDR2 and CDR3, respectively. The length of an L chain CDR1 can vary substantially between different VL regions. For example, the length of CDR1 can vary from about 7 amino acids to about 17 amino acids. In contrast, the lengths of L chain CDR2 and CDR3 typically do not vary between different VL regions. The length of an H chain CDR3 can vary substantially between different VH regions. For example, the length of CDR3 can vary from about 1 amino acid to about 20 amino acids. Each H and L chain CDR region is flanked by FR regions.

Limited digestion of an immunoglobulin with a protease may produce two fragments. An antigen binding fragment is referred to as an Fab, an Fab', or an F(ab')₂ fragment. A fragment lacking the ability to bind to antigen is referred to as an Fc fragment. A Fab fragment comprises one arm of an immunoglobulin molecule containing a L chain (VL+CL domains) paired with the VH region and a portion of the CH region (CHI domain). An Fab' fragment corresponds to an Fab fragment with part of the hinge region attached to the CHI domain. An F(ab')₂ fragment corresponds to two Fab' fragments that are normally covalently linked to each other through a disulfide bond, typically in the hinge regions.

Isolated antibodies of the present invention can include serum containing such antibodies, or antibodies that have been purified to varying degrees. Whole antibodies of the present invention can be polyclonal or monoclonal. Alternatively, functional equivalents of whole antibodies, such as antigen binding fragments in which one or more antibody domains are truncated or absent (e.g., Fv, Fab, Fab', or F(ab')₂ fragments), as well as genetically-engineered antibodies or antigen binding fragments thereof, including single chain antibodies (e.g., scFv), humanized antibodies, antibodies that can bind to more than one epitope (e.g., bi-specific antibodies), or antibodies that can bind to one or more different antigens (e.g., bi- or multi- specific antibodies), may also be employed in the invention.

In some embodiments, the targeting portion comprises an antibody. In some embodiments, the targeting moiety is a scFv. In some embodiments, the targeting portion is a scFv comprising a (i) a light chain variable domain of SEQ ID NO: 13; and/or (ii) heavy chain variable domain of SEQ ID NO: 15. In some embodiments, the targeting portion is a scFv comprising (i) a light chain variable domain of SEQ ID NO: 14; and/or (ii) heavy chain variable domain of SEQ ID NO: 16. In some embodiments, the targeting portion is a scFv having the sequence of SEQ ID NO: 17. In some embodiments, the targeting portion is a scFv having the sequence of SEQ ID NO: 18.

In some embodiments, the targeting portion is a scFv comprising a (i) a light chain variable domain of SEQ ID NO:34; and/or (ii) heavy chain variable domain of SEQ ID NO:36. In some embodiments, the targeting portion is a scFv comprising (i) a light chain variable domain of SEQ ID NO:35; and/or (ii) heavy chain variable domain of SEQ ID NO:36. In some embodiments, the targeting portion is a scFv having the sequence of SEQ ID NO:37. In some embodiments, the targeting portion is a scFv having the sequence of SEQ ID NO:38.

In one embodiment, targeted molecules of the present invention include humanized antibodies or a fragment thereof (such as a humanized scFv). A humanized antibody or fragment thereof are molecules having an antigen binding site derived from an immunoglobulin from a non-human species, the remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site may comprise either complete variable regions fused onto human constant domains or only the complementarity determining regions (CDRs) grafted onto appropriate human framework regions in the variable domains. A humanized antibody or fragment thereof can be produced, for example, by modeling the antibody variable domains, and producing the antibodies using genetic engineering techniques, such as CDR grafting. A description of various techniques for the production of humanized antibodies is found, for example, in Morrison et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-55; Whittle et al. (1987) Prot. Eng. 1:499-505; Co et al. (1990) J. Immunol. 148: 1149-1154; Co et al. (1992) Proc. Natl. Acad. Sci. USA 88:2869-2873; Carter et al. (1992) Proc. Natl. Acad. Sci. 89:4285-4289; Routledge et al. (1991) Eur. J. Immunol. 21:2717-2725 and PCT Patent Publication Nos. WO 91/09967; WO 91/09968 and WO 92/113831.

In some embodiments, the antibody or fragment thereof does not activate complement activation. Methods of modifying antibodies or fragments thereof by reducing or eliminating their complement activation activities are known in the art (Tan et al. (1990) Proc Natl Acad Sci USA 87, 162-166).

5 In some embodiments, the targeting portion of the targeted molecules is a homolog of any of the targeting portion amino acid sequences described herein or a biologically active fragment thereof. Homologs of the targeting portion (or biologically active fragments thereof) include proteins which differ from a targeting portion described herein (or biologically-active fragment thereof) in that at least one or a few, but not
10 limited to one or a few, amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide or fragment), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation, amidation and/or addition glycosylphosphatidyl inositol). For example, homologue of a targeting portion may have an amino acid sequence that is at least about
15 70% identical to the amino acid sequence of a targeting portion described herein, for example at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of a targeting portion described herein. Amino acid sequence identity can be determined in various ways, for example, using publicly
20 available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGNTM (DNAST AR) software. One skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

The molecules described herein in some embodiments comprise an
25 inhibitor portion comprising a complement modulator, such as a complement inhibitor.

As used herein, the term "complement inhibitor" refers to any compound, composition, or protein that reduces or eliminates complement activity. The reduction in complement activity may be incremental (e.g., a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% reduction in activity) or complete. For example, in some embodiments, a
30 complement inhibitor can inhibit complement activity by at least 10 (e.g., at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 or greater) % in a standard in

vitro red blood cell hemolysis assay or an in vitro CH50eq assay. See, e.g., Kabat and Mayer (eds), "Experimental Immunochemistry, 2nd Edition," 135-240, Springfield, IL, CC Thomas (1961), pages 135-139, or a conventional variation of that assay such as the chicken erythrocyte hemolysis method as described in, e.g., Hillmen et al. (2004) N Engl J Med 350(6):552.

The CH50eq assay is a method for measuring the total classical complement activity in serum. This test is a lytic assay, which uses antibody-sensitized erythrocytes as the activator of the classical complement pathway and various dilutions of the test serum to determine the amount required to give 50% lysis (CH50). The percent hemolysis can be determined, for example, using a spectrophotometer. The CH50eq assay provides an indirect measure of terminal complement complex (TCC) formation, since the TCC themselves are directly responsible for the hemolysis that is measured.

The assay is well known and commonly practiced by those of skill in the art. Briefly, to activate the classical complement pathway, undiluted serum samples (e.g., human serum samples) are added to microassay wells containing the antibody-sensitized erythrocytes to thereby generate TCC. Next, the activated sera are diluted in microassay wells, which are coated with a capture reagent (e.g., an antibody that binds to one or more components of the TCC). The TCC present in the activated samples bind to the monoclonal antibodies coating the surface of the microassay wells. The wells are washed and, to each well, is added a detection reagent that is detectably labeled and recognizes the bound TCC. The detectable label can be, e.g., a fluorescent label or an enzymatic label. The assay results are expressed in CH50 unit equivalents per milliliter (CH50 U Eq/mL).

Additional methods for detecting and/or measuring complement activity in vitro are set forth and exemplified in the working examples.

The complement inhibitor described herein in some embodiments is a specific inhibitor of the lectin pathway. In some embodiments, the complement inhibitor is a specific inhibitor of the alternative pathway. In some embodiments, the complement inhibitor is a specific inhibitor of the classical pathway.

In some embodiments, the complement inhibitor is a soluble or membrane-bound protein such as, for example, membrane cofactor protein (MCP), decay

accelerating factor (DAF/CD55), CD59, mouse complement receptor 1-related gene/protein y (Crry), human complement receptor 1 (CRI) or factor H, or Factor I, or an antibody specific for a component of a complement pathway such as, for example, eculizumab (an anti-CS antibody marketed under the trade name Soliris®), pexelizumab (the antigen-binding fragment of eculizumab), an anti-factor B antibody (such as the monoclonal antibody 1379 produced by ATCC Deposit No. PTA-6230), an anti-properdin antibody, an anti-factor D antibody, an anti-MASP antibody, an anti MB L-antibody, and the like (see below). Alternatively, a complement inhibitor may be a small molecule or a linear or cyclic peptide such as, for example, compstatin, N-acetylaspartylglutamic acid (NAAGA), and the like. In some embodiments, the complement inhibitor is selected from the group consisting of: an anti-C5 antibody, an Eculizumab, an pexelizumab, an anti-C3b antibody, an anti-C6 antibody, an anti-C7 antibody, an anti- factor B antibody, an anti- factor D antibody, and an anti-properdin antibody, a human membrane co factor protein (MCP), a human decay accelerating factor (DAF), a mouse decay accelerating factor (DAF), a human CD59, a mouse CD59, a mouse CD59 isoform B, a mouse Crry, a human CR1, a Factor I, a human factor H, a mouse factor H, and a biologically active fragment of any the preceding.

As used herein, the term "membrane cofactor protein," "MCP," or "CD46" refers to a widely distributed C3b/C4b-binding cell surface glycoprotein which inhibits complement activation on host cells and serves as a cofactor for the factor I-mediated cleavage of C3b and C4b, including homologs thereof. T.J. Oglesby et al., J. Exp. Med. (1992) 175: 1547-1551. MCP belongs to a family known as the regulators of complement activation ("RCA"). Family members share certain structural features, comprising varying numbers of short consensus repeat (SCR) domains, which are typically between 60 and 70 amino acids in length. Beginning at its amino-terminus, MCP comprises four SCRs, a serine/threonine/proline-enriched region, an area of undefined function, a transmembrane hydrophobic domain, a cytoplasmic anchor and a cytoplasmic tail. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that human MCP or biologically active fragments thereof encompass all species and strain variations.

SEQ ID NO:44 represents the full-length human MCP amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. P15529). Amino acids 1-34 correspond to the signal peptide, amino acids 35-343 correspond to the extracellular domain, amino acids 344-366 correspond to the transmembrane domain, and amino acids 367-392 correspond to the cytoplasmic domain. In the extracellular domain, amino acids 35-96 correspond to SCR 1, amino acids 97-159 correspond to SCR 2, amino acids 160-225 correspond to SCR 3, amino acids 226-285 correspond to SCR 4, and amino acids 302-326 correspond to the serine/threonine-rich domain. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that MCP or biologically active fragments thereof encompass all species and strain variations. As used herein, the term "biologically active" fragment of MCP refers to any soluble fragment lacking both the cytoplasmic domain and the transmembrane domain, including fragments comprising, consisting essentially of or consisting of 1, 2, 3, or 4 SCR domains, with or without the serine/threonine-rich domain, having some or all of the complement inhibitory activity of the full-length human MCP protein. In some embodiments, the complement inhibitor portion comprises full-length human MCP (amino acids 35-392 of SEQ ID NO:44), the extracellular domain of human MCP (amino acids 35-343 of SEQ ID NO:44), or SCRs 1-4 of human MCP (amino acids 35-285 of SEQ ID NO:44).

Decay accelerating factor, also referred to as CD55 (DAF/CD55) (SEQ ID NO:45 and SEQ ID NO:46), is an ~70 kilodalton (kDa) membrane-bound glycoprotein which inhibits complement activation on host cells. Like several other complement regulatory proteins, DAF comprises several approximately 60 amino acid repeating motifs termed short consensus repeats (SCR).

As used herein, the term "decay accelerating factor," "DAF," or "CD55" refers to a seventy kilodalton ("kDa") membrane glycoprotein comprising four short consensus repeat (SCR) domains followed by a heavily O-glycosylated serine/threonine-rich domain at the C-terminus that elevates the molecule from the membrane surface, followed by a glycosylphosphatidylinositol ("GPI") anchor. DAF protects the cell surface from complement activation by dissociating membrane-bound C3 convertases that are required to cleave complement protein C3 and to amplify the complement cascade. DAF

prevents assembly or accelerates decay of both the C3- and C5-convertases of the alternative and classical complement pathways.

SEQ ID NO:45 represents the full-length human DAF amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. P08173); SEQ ID NO:46
5 represents the full-length mouse DAF amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. Q61475). In the human DAF sequence, amino acids 1-34 correspond to the signal peptide, amino acids 35-353 appear in the mature protein, and amino acids 354-381 are removed from the polypeptide after translation. Within the mature protein, amino acids 35-96 correspond to SCR 1, amino acids 96- 160 correspond to SCR 2,
10 amino acids 161-222 correspond to SCR 3, amino acids 223-285 correspond to SCR 4, and amino acids 287-353 correspond to the O-glycosylated serine/threonine-rich domain. The GPI anchor is attached to human DAF at a serine at position 353. In the mouse DAF sequence, amino acids 1-34 correspond to the signal peptide, amino acids 35-362 appear in the mature protein, and amino acids 363-390 are removed from the polypeptide after
15 translation. Within the mature protein, amino acids 35-96 correspond to SCR 1, amino acids 97-160 correspond to SCR 2, amino acids 161-222 correspond to SCR 3, amino acids 223-286 correspond to SCR 4, and amino acids 288-362 correspond to the O-glycosylated serine/threonine-rich domain. The GPI anchor is attached to mouse DAF at a serine at position 362. It is understood that species and strain variations exist for the
20 disclosed peptides, polypeptides, and proteins, and that DAF or biologically active fragments thereof encompass all species and strain variations. As used herein, the term "biologically active" fragment of DAF refers to any fragment of DAF lacking a GPI anchor and/or the amino acid to which it is attached (i.e., Ser-353), including any fragments of the full-length DAF protein comprising, consisting essentially of or
25 consisting of 1, 2, 3, or 4 SCR domains, with or without the O-glycosylated serine/threonine-rich domain, having some or all the complement inhibitory activity of the full-length DAF protein.

As used herein, the term "CD59" refers to a membrane-bound 128 amino acid glycoprotein that potently inhibits the membrane attack complex (MAC) of
30 complement. CD59 acts by binding to the C8 and/or C9 components of the MAC during assembly, ultimately preventing incorporation of the multiple copies of C9 required for

complete formation of the osmolytic pore at the heart of the MAC. CD59 is both N- and O-glycosylated. The N-glycosylation comprises primarily bi- or tri-antennary structures with and without lactosamine and outer arm fucose residues, with variable sialylation present at some sites. Like DAF, CD59 is anchored in the cell membrane by a

5 glycosylphosphatidylinositol ("GPI") anchor, which is attached to an asparagine at amino acid 102. Soluble forms of CD59 (sCD59) have been produced, but they generally have low functional activity in vitro, particularly in the presence of serum, suggesting that unmodified sCD59 has little or no therapeutic efficacy. See, e.g., S. Meri et al., "Structural composition and functional characterization of soluble CD59: heterogeneity

10 of the oligosaccharide and glycosphosphoinositol (GPI) anchor revealed by laser-desorption mass spectrometric analysis," *Biochem. J.* 316:923-935 (1996).

SEQ ID NO:47 represents the full-length human CD59 amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. P13987); SEQ ID NO:48 represents the full-length mouse CD59 sequence, isoform A (see, e.g., UniProtKB/Swiss-

15 Prot. Accession No. 055186); SEQ ID NO:49 represents the full-length mouse CD59 sequence, isoform B (see, e.g., UniProtKB/SwissProt. Accession No. P58019). In the human CD59 sequence, amino acids 1-25 of SEQ ID NO:47 correspond to the leader peptide, amino acids 26-102 of SEQ ID NO:47 correspond to the mature protein, and amino acids 103-128 of SEQ ID NO:47 are removed after translation. The GPI anchor is

20 attached to CD59 at an asparagine at position 102 of SEQ ID NO:47. In isoform A of the mouse CD59 sequence, amino acids 1-23 of SEQ ID NO:48 correspond to the leader peptide, amino acids 24-96 of SEQ ID NO: 48 correspond to the mature protein, and amino acids 97-123 of SEQ ID NO:48 are removed after translation. The GPI anchor is attached to CD59 at a serine at position 96 of SEQ ID NO: 48. In isoform B of the mouse

25 CD59 sequence, amino acids 1-23 of SEQ ID NO: 49 correspond to the leader peptide, amino acids 24-104 of SEQ ID NO: 49 correspond to the mature protein, and amino acids 105-129 of SEQ ID NO:49 are removed after translation. The GPI anchor is attached to CD59 at an asparagine at position 104 of SEQ ID NO:49. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that

30 CD59 or biologically active fragments thereof encompass all species and strain variations.

As used herein, the term "biologically active" fragment of human CD59 refers to any fragment of human CD59 lacking a GPI anchor and/or the amino acid to which it is attached (i.e., Asn-102), including any fragments of the full-length human CD59 protein having some or all the complement inhibitory activity of the full-length
 5 CD59 protein; and the term "biologically active" fragment of mouse CD59 refers to any fragment of mouse CD59 isoform A or isoform B lacking a GPI anchor and/or the amino acid to which it is attached (i.e., Ser-96 of isoform A, or Asp- 104 of isoform B), including any fragments of either full-length mouse CD59 protein isoform having some or all the complement inhibitory activity of the full-length CD59 protein.

10 As used herein, the term "mouse complement receptor 1 -related gene/protein y" or "Crry" refers to a membrane-bound mouse glycoprotein that regulates complement activation, including homologs thereof. Crry regulates complement activation by serving as a cofactor for complement factor I, a serine protease which cleaves C3b and C4b deposited on host tissue. Crry also acts as a decay- accelerating
 15 factor, preventing the formation of C4b2a and C3bBb, the amplification convertases of the complement cascade.

SEQ ID NO:50 represents the full-length mouse Crry protein amino acid sequence. Amino acids 1-40 correspond to the leader peptide, amino acids 41-483 of SEQ ID NO:50 correspond to the mature protein, comprising amino acids 41-405 of
 20 ID NO:50, corresponding to the extracellular domain, amino acids 406-426 of SEQ ID NO:50, corresponding to the transmembrane domain, and amino acids 427-483 of SEQ ID NO:50, corresponding to the cytoplasmic domain. In the extracellular domain, amino acids 83-143 of SEQ ID NO:50 correspond to SCR 1, amino acids 144-205 of SEQ ID NO:50 correspond to SCR 2, amino acids 206-276 of SEQ ID NO:50 correspond to SCR
 25 3, amino acids 277-338 of SEQ ID NO:50 correspond to SCR 4, and amino acids 339-400 of SEQ ID NO:50 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 encompasses all species and strain variations. As used herein, the term "biologically active" fragment of mouse Crry protein
 30 refers to any soluble fragment of mouse 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 mouse Crry protein having some or all the complement inhibitory activity of the full-length Crry protein. In one embodiment, the biologically active fragment of mouse Crry comprises amino acids 85-403 of SEQ ID NO: 50.

5 As used herein, the term "complement receptor 1," "CR1," or "CD35" refers 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 is expressed principally on erythrocytes, monocytes, neutrophils, and B cells, but is also present on some T lymphocytes, mast cells, and glomerular podocytes. CR1 protein is typically expressed at between 100 and 1000 copies per cell. CR1 is the main system for processing and clearance of complement-opsonized immune complexes. CR1 negatively regulates the complement cascade, mediates immune adherence and phagocytosis, and inhibits both the classic and alternative complement pathways. The full-length CR1 protein comprises 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 has 25 potential N-glycosylation signal sequences, and comprises 30 short consensus ("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 ranges between 60-99 percent. The 30 SCR domains are further 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. The first three comprise seven SCR domains each, while LHR-D comprises 9 SCR domains. The active sites on the extracellular domain of CR1 protein include a C4b-binding site with lower affinity for C3b in SCRs 1-4 comprising amino acids 42-295, 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 Clq-binding site in SCRs 22-28 comprising amino acids 1394-1842.

30 SEQ ID NO:51 represents the full-length human CR1 amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. P17927). Amino acids 1-41 correspond to the signal peptide, amino acids 42-2039 correspond to the mature protein, comprising

amino acids 42-1971, corresponding to the extracellular domain, amino acids 1972-1996, corresponding to the transmembrane domain, and amino acids 1997-2039, corresponding to the cytoplasmic domain. In the extracellular domain, amino acids 42-101 correspond to SCR 1, 102-163 correspond to SCR2, amino acids 164-234 correspond to SCR3, amino acids 236-295 correspond to SCR4, amino acids 295-355 correspond to SCR5, amino acids 356-418 correspond to SCR6, amino acids 419-489 correspond to SCR7, amino acids 491-551 correspond to SCR8, amino acids 552- 613 correspond to SCR9, amino acids 614-684 correspond to SCR10, amino acids 686-745 correspond to SCR11, amino acids 745-805 correspond to SCR12, amino acids 806-868 correspond to SCR13, amino acids 869-939 correspond to SCR 14, amino acids 941-1001 correspond to SCR15, amino acids 1002-1063 correspond to SCR16, amino acids 1064-1134 correspond to SCR17, amino acids 1136-1195 correspond to SCR18, amino acids 1195-1255 correspond to SCR 19, amino acids 1256-1318 correspond to SCR 20, amino acids 1319-1389 correspond to SCR 21, amino acids 1394-1454 correspond to SCR 22, amino acids 1455-1516 correspond to SCR 23, amino acids 1517-1587 correspond to SCR 24, amino acids 1589-1648 correspond to SCR 25, amino acids 1648-1708 correspond to SCR 26, amino acids 1709-1771 correspond to SCR 27, amino acids 1772-1842 correspond to SCR 28, amino acids 1846-1906 correspond to SCR 29, amino acids 1907-1967 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 encompass all species and strain variations. As used herein, the term "biologically active" fragment of CR1 protein refers 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.

As used herein, the term "complement factor H," "factor H," or "FH" refers to complement factor H, a single polypeptide chain plasma glycoprotein, including homologs thereof. The protein is 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 binds to C3b, accelerates the decay of the alternative pathway C3-convertase (C3bBb), and acts as a cofactor for the proteolytic inactivation of C3b. In the presence of factor H, proteolysis by factor I results in the cleavage and inactivation of C3b. Factor H has at least three distinct binding domains for C3b, which are located within SCRs 1-4, SCRs 5-8, and SCRs 19-20. Each domain binds to a distinct region within the C3b protein: the N-terminal sites bind to native C3b; the second site, located in the middle region of factor H, binds to the C3c fragment and the site located within SCR19 and 20 binds to the C3d region. In addition, factor H also contains binding sites for heparin, which are located within SCR 7, SCRs 5-12, and SCR 20 of factor H and 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 are located within the first four N-terminal SCR domains.

SEQ ID NO:52 represents the full-length human factor H amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. P08603); SEQ ID NO:53 represents the full-length mouse factor H amino acid sequence (see, e.g., UniProtKB/Swiss-Prot. Accession No. P06909). In the human factor H sequence, amino acids 1-18 of SEQ ID NO:52 correspond to the signal peptide, and amino acids 19-1231 of SEQ ID NO:52 correspond to the mature protein. Within that protein, amino acids 21-80 of SEQ ID NO:52 correspond to SCR 1, amino acids 85-141 of SEQ ID NO:52 correspond to SCR 2, amino acids 146-205 of SEQ ID NO:52 correspond to SCR 3, amino acids 210-262 of SEQ ID NO:52 correspond to SCR 4, and amino acids 267-320 of SEQ ID NO:52 correspond to SCR 5. In the mouse factor H sequence, amino acids 1-18 of SEQ ID NO:53 correspond to the signal peptide, and amino acids 19-1234 of SEQ ID NO:53 correspond to the mature protein. Within that protein, amino acids 19-82 of SEQ ID NO:53 correspond to SCR 1, amino acids 83-143 of SEQ ID NO:53 correspond to SCR 2, amino acids 144-207 of SEQ ID NO:53 correspond to SCR 3, amino acids 208-264 of SEQ ID NO:53 correspond to SCR 4, and amino acids 265-322 of SEQ ID NO:53 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 encompass all species and strain variations.

As used herein, the term "biologically active" fragment of factor H refers to any portion of a factor H protein having some or all the complement inhibitory activity of the full-length factor H protein, and includes, 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 embodiments, the biologically active fragment of factor H has one or more of the following properties: (1) binding to C-reactive protein (CRP), (2) binding to C3b, (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) C3b decay- acceleration activity, and (10) inhibiting the alternative complement pathway.

SEQ ID NO: 59 represents the amino acid sequence for mannose-binding lectin-associated protein of 44kDa (MAp44). MAp44 is an alternatively spliced product encoded by the MASP1 gene. In certain aspects, MAp44 is an inhibitor of lectin pathway activation.

Thus, in some embodiments, the inhibitor portion of the targeted molecule described herein comprises a complement inhibitor or biologically active fragment thereof. In some embodiments, the complement inhibitor is selected from the group consisting of human MCP, human DAF, mouse DAF, human CD59, mouse CD59 isoform A, mouse CD59 isoform B, mouse Crry protein, human CR1, human factor H, or mouse factor H, a Factor I, MAp44 or a biologically active fragment thereof.

In some embodiments, the inhibitor portion comprises full-length human MCP (SEQ ID NO:44). In some embodiments, the complement inhibitor portion of the targeting construct comprises a biologically active fragment of human MCP (SEQ ID NO:44). In some embodiments, the biologically active fragment of human MCP is selected from the group consisting of SCRs 1-4 (amino acids 35-285 of SEQ ID NO:44), SCRs 1-4 plus the serine/threonine-rich domain (amino acids 35-326 of SEQ ID NO:44), and the extracellular domain of MCP (amino acids 35-343 of SEQ ID NO:44).'

In some embodiments, the inhibitor portion comprises full-length human DAF. In some embodiments, the inhibitor portion comprises a biologically active fragment of human DAF (SEQ ID NO:45). In some embodiments, the biologically active

fragment of human DAF is selected from the group consisting of SCRs 1-4 (amino acids 25-285 of SEQ ID NO:45) and SCRs 1-4 plus the O- glycosylated serine/threonine-rich domain (amino acids 25-353 of SEQ ID NO:45). In some embodiments, the inhibitor portion comprises full-length mouse DAF (SEQ ID NO:46). In some embodiments, the inhibitor portion comprises a biologically active fragment of mouse DAF. In some
 5 embodiments, the biologically active fragment of mouse DAF is selected from the group consisting of SCRs 1-4 (amino acids 35-286 of SEQ ID NO:46) and SCRs 1-4 plus the O-glycosylated serine/threonine-rich domain (amino acids 35-362 of SEQ ID NO:46).

 In some embodiments, the inhibitor portion comprises full-length human
 10 CD59 (SEQ ID NO:47). In some embodiments, the inhibitor portion comprises a biologically active fragment of human CD59 (SEQ ID NO:47). In some embodiments, the biologically active fragment of human CD59 comprises the extracellular domain of human CD59 lacking its GPI anchor (amino acids 26-101 of SEQ ID NO:47). In some embodiments, the inhibitor portion comprises full-length mouse CD59, isoform A (SEQ
 15 ID NO:48). In some embodiments, the inhibitor portion comprises a biologically active fragment of mouse CD59, isoform A (SEQ ID NO:48). In some embodiments, the biologically active fragment of mouse CD59, isoform A comprises the extracellular domain of mouse CD59, isoform A lacking its GPI anchor (amino acids 24-95 of SEQ ID NO:48). In some embodiments, the inhibitor portion comprises full-length mouse CD59,
 20 isoform B (SEQ ID NO:49). In some embodiments, the c inhibitor portion comprises a biologically active fragment of mouse CD59, isoform B (SEQ ID NO:49). In some embodiments, the biologically active fragment of mouse CD59, isoform B comprises the extracellular domain of mouse CD59, isoform lacking its GPI anchor (amino acids 24-103 of SEQ ID NO:49).

 In some embodiments, the inhibitor portion comprises full-length mouse
 25 Crry protein (SEQ ID NO:50). In some embodiments, the inhibitor portion comprises a biologically active fragment of mouse Crry protein (SEQ ID NO:50). In some embodiments, the biologically active fragment of mouse Crry protein is selected from the group consisting of SCRs 1-5 (amino acids 41-400 of SEQ ID NO:50) and the
 30 extracellular domain of mouse Crry protein (amino acids 41-405 of SEQ ID NO:50). In

one embodiment, the inhibitor portion comprises the biologically active fragment of mouse Crry comprising amino acids 85-403 of SEQ ID NO: 50.

In some embodiments, the inhibitor portion comprises full-length human CR1 protein (SEQ ID NO:51). In some embodiments, the inhibitor portion comprises a biologically active fragment of human CR1 protein (SEQ ID NO:51). In some
5 embodiments, the biologically active fragment of human CR1 protein is selected from the group consisting of SCRs 1-4 (amino acids 42-295 of SEQ ID NO:51), SCRs 1-10 (amino acids 42-684 of SEQ ID NO:51), SCRs 8-11 (amino acids 490-745 of SEQ ID NO:51), SCRs 15-18 (amino acids 940-1196 of SEQ ID NO:51), and SCRs 22-28 (amino
10 acids 1394-1842 of SEQ ID NO:51).

In some embodiments, the inhibitor portion comprises full-length human (SEQ ID NO:52) or mouse (SEQ ID NO:53) factor H. In some embodiments, the inhibitor portion comprises a biologically active fragment of human (SEQ ID NO:52) or mouse (SEQ ID NO:53) factor H. In some embodiments, the biologically active fragment
15 of human factor H (SEQ ID NO:52) is selected from the group consisting of SCRs 1-4 (amino acids 21-262 of SEQ ID NO:52), SCRs 1-5 of factor H (amino acids 21-320 of SEQ ID NO:52), SCRs 1-8 of factor H (amino acids 21-507 of SEQ ID NO:52), and SCRs 1-18 of factor H (amino acids 21-1104 of SEQ ID NO:52). In some embodiments, the biologically active fragment of mouse factor H (SEQ ID NO:53) is selected from the
20 group consisting of SCRs 1-4 (amino acids 19-264 of SEQ ID NO:53), SCRs 1-5 of factor H (amino acids 19-322 of SEQ ID NO:53), SCRs 1-8 of factor H (amino acids 19-507 of SEQ ID NO:53), and SCRs 1-18 of factor H (amino acids 19-1109 of SEQ ID NO:53). In some embodiments, the biologically active fragment of human (SEQ ID NO:52) or mouse (SEQ ID NO:53) factor H comprises (and in some embodiments
25 consists of or consists essentially of) at least the first four N-terminal SCR domains of factor H, including for example, at least any of the first 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or more N-terminal SCR domains of factor H.

In some embodiments, the inhibitor portion comprises MAp44 (SEQ ID NO:59). In some embodiments, the inhibitor portion comprises a biologically active
30 fragment of MAp44 (SEQ ID NO: 59).

In some embodiments, the inhibitor portion of the targeted molecules is a homolog of any of the complement inhibitors described herein or a biologically active fragment thereof. Homologs of the complement inhibitors (or biologically active fragments thereof) include proteins which differ from a naturally occurring complement inhibitor (or biologically-active fragment thereof) in that at least one or a few, but not limited to one or a few, amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide or fragment), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation, amidation and/or addition glycosylphosphatidyl inositol). For example, 5
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homologue of a complement inhibitor may have an amino acid sequence that is at least about 70% identical to the amino acid sequence of a naturally complement inhibitor (e.g., SEQ ID NOs:44-53), for example at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of a naturally occurring complement inhibitor (e.g., SEQ ID NOs:44-53, 59). Amino acid sequence identity can be determined in various ways, for example, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNAST AR) software. One skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

In certain embodiments, a homolog of complement inhibitor (or a biologically active fragment thereof) retains all the complement pathway inhibitory activity of the complement inhibitor (or a biologically active fragment thereof) from which it is derived. In certain embodiments, the homolog of a complement inhibitor (or a biologically-active fragment thereof) retains at least about 50%, for example, at least 25
about any of 60%, 70%, 80%, 90%, or 95% of the complement inhibition activity the complement inhibitor (or a biologically-active fragment thereof) from which is derived.

In some embodiments, the inhibitor portion comprises an antibody (or an antigen binding fragment thereof) that binds to a complement component, e.g., a 30
complement component selected from the group consisting of C1, C1q, C1s, C2, C2a, C3, C3a, C3b, C4, C4b, C5, C5a, C5b, C6, C7, C8, and C9. The complement polypeptides to

which the antibodies or antigen binding fragments thereof bind can be, in some embodiments, human polypeptides, e.g., human C1, C1q, C1s, C2, C2a, C3, C3a, C3b, C4, C4b, C5, C5a, C5b, C6, C7, C8, C9, factor B, factor D, or properdin polypeptides. The amino acid sequences for the foregoing complement proteins are well-known in the art as
5 are methods for preparing the proteins or fragments thereof for use in preparing an antibody (or antigen-binding fragment thereof) specific for one or more of the complement proteins. Suitable methods are also described and exemplified herein.

Exemplary anti-complement protein antibodies, which are suitable for incorporation into the targeted molecules described herein and for subsequent use in any
10 of the methods described herein, are also well known in the art. For example, antibodies that bind to complement component C5 and inhibit the cleavage of C5 into fragments C5a and C5b include, e.g., eculizumab (Soliris®; Alexion Pharmaceuticals, Inc., Cheshire, CT) and pexelizumab (Alexion Pharmaceuticals, Inc., Cheshire, CT). See, e.g., Kaplan (2002) *Curr Opin Investig Drugs* 3(7): 1017-23; Hill (2005) *Clin Adv Hematol Oncol* 3(11):849-50; Rother et al. (2007) *Nature Biotechnol* 25(11): 1256-1488; Whiss (2002) *Curr Opin Investig Drugs* 3(6):870-7; Patel et al. (2005) *Drugs Today (Bare)* 41(3): 165-70; and Thomas et al. (1996) *Mol Immunol* 33(17- 18): 1389-401.
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In some embodiments, the anti-C5 antibody can bind to an epitope in the alpha chain of the human complement component C5 protein. Antibodies that bind to the
20 alpha chain of C5 are described in, for example, PCT application publication no. WO 2010/136311 and U.S. patent no. 6,355,245. In some embodiments, the anti-C5 antibody can bind to an epitope in the beta chain of the human complement component C5 protein. Antibodies that bind to the C5 beta chain are described in, e.g., Moongkarndi et al. (1982) *Immunobiol* 162:397; Moongkarndi et al. (1983) *Immunobiol* 165:323; and Mollnes et al. (1988) *Scand J Immunol* 28:307-312.
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Additional anti-C5 antibodies, and antigen-binding fragments thereof, suitable for use in the targeting constructs described herein are described in, e.g., PCT application publication no. WO 2010/015608, the disclosure of which is incorporated herein by reference in its entirety.

30 Antibodies that bind to C3b and, for example, inhibit the C3b convertase are also well known in the art. For example, PCT application publication nos. WO

2010/136311, WO2009/056631, and WO 2008/154251, the disclosures of each of which are incorporated herein by reference in their entirety. Antagonistic anti-C6 antibodies and anti-C7 antibodies have been described in, e.g., Brauer et al.(1996) Transplantation 61(4):S88-S94 and U.S. patent no. 5,679,345.

5 In some embodiments, the inhibitor portion comprises an anti-factor B antibody (such as the monoclonal antibody 1379 produced by ATCC Deposit No. PTA-6230). Anti-factor B antibodies are also described in, e.g., Ueda et al. (1987) J Immunol 138(4): 1143-9; Tanhehco et al. (1999) Transplant Proc 31(5):2168-71; U.S. patent application publication nos. 20050260198 and 2008029911; and PCT publication no. WO
10 09/029669.

 In some embodiments, the inhibitor portion comprises an anti-factor D antibody, e.g., an antibody described in Pascual et al. (1990) J Immunol Methods 127:263-269; Sahu et al. (1993) Mol Immunol 30(7):679-684; Pascual et al. (1993) Eur J Immunol 23: 1389-1392; Niemann et al. (1984) J Immunol 132(2):809-815; U.S. patent
15 no. 7,439,331; or U.S. patent application publication no. 20080118506.

 In some embodiments, the inhibitor portion comprises an anti-properdin antibody. Suitable anti-properdin antibodies are also well-known in the art and include, e.g., U.S. patent application publication nos. 20110014614 and PCT application publication no. W02009110918.

20 In some embodiments, the inhibitor portion comprises an anti-MBL antibody. Mannose-binding mannan-binding lectin (MBL), a plasma protein, forms a complex with proteins known as MBL-associated serine proteases (MASPs). MBL binds to several monosaccharides that are uncharacteristic of mammalian proteins, e.g., mannose, N-acetylglucosamine, N-acetylmannoseamine, L-fucose and glucose, whereas
25 sialic acid and galactose are not bound. When the MBL-MASP complex binds to microorganisms, the proenzymic forms of the serine proteases are activated and mediate the activation of complement components C4 and C2, thereby generating the C3 convertase C4b2b and leading to opsonization by the deposition of C4b and C3b fragments. MASP -2 has been shown to cleave C4 and C2, while MASP-1 may be
30 responsible for direct cleavage of C3. The functions of MASP-3 and MAp19 are less well understood. Studies have shown a clear link between low levels of MBL and opsonic

deficiency, as well as clinical manifestations such as severe diarrhea, chronic hepatitis and HIV infection, and autoimmune disease. See, Petersen et al., *J. Immunological Methods*, 257: 107-16 (2001); Petersen et al., *Molecular Immunology*, 38: 133-49 (2001). Anti-mannan-binding lectin antibodies are known in the art (see, e.g., Pradhan et al. (2012) *Rheumatol. Int.* epublished September, 2012) and commercially available (AbCam).

In some embodiments, the inhibitor portion comprises an anti-MASP antibody. The mannan-binding lectin-associated serine proteases (MASPs) are a family of at least three proteins (mannan-binding lectin-associated serine protease- 1, -2 and -3 (MASP-1, MASP-2 and MASP-3, respectively)), which have been taught to play a significant role in modulation of the lectin pathway of complement activation. Petersen et al., *Molecular Immunology* 38: 133-149 (2001).

MASP-1 has a histidine loop structure of the type found in trypsin and trypsin-like serine proteases. MASP-1 has been found to be involved in complement activation by MBL. A cDNA clone encoding MASP-1 has been reported that encodes a putative leader peptide of 19 amino acids followed by 680 amino acid residues predicted to form the mature peptide. MASP-2 (MBL-associated serine protease 2) is a serine protease also similar in structure to C1r and C1s of the complement pathway. Like these, and contrary to MASP-1, it has no histidine loop structure of the type found in trypsin and trypsin-like serine proteases. It has been theorized that MASP-1 can cleave C3, generating C3b, which may be deposited on an activated cell or tissue surface

It has been shown that MASP-2, cleaves C4 and C2, giving rise to the C3 convertase, C4b2b (Thiel et al., *Nature*, 386:506-10 (1997)). The MASP-2 protein comprises of a number of domains namely the CUB1, EGF, CUB2, CCPI, CCP2 and serine protease domains. It is believed that the domain responsible for association with MBL is situated in the N-terminus, whereas the serine protease domain is responsible for the serine protease activity of MASP-2. sMAP, also known as MAP19, is a 19 kd is derived from the same gene as MASP-2, which lacks the serine protease domain and a major part of the A chain. Skjoedt et al., *Immunobiology*, 215:921-31 (2010). Recently, a third member of the family, MASP-3 was identified, which shares a high degree of

homology with MASP-1, such that it appears that MASP-1 and MASP-3 are generated as a result of alternative splicing of primary mRNA transcripts.

Antibodies against MBL, MASP- 1 , MASP-2, MASP-3 and the MBL/MASP complex, and their use for inhibiting the adverse effects of complement activation, such as ischemia-reperfusion injury, have been disclosed, for example, in
5 WO04/075837; US 2009/0017031.

Other antibodies to MASP-2 have been described previously, as well. See, e.g., WO 02/06460, US2007/0009528, Peterson et al., Mol. Immunol. 37:803-11 (2000), MoUer-Kristensen et al., J. of Immunol. Methods 282: 159-67 (2003), Petersen et al.,
10 Mol. Immunol. 35:409, and WO 04/106384.

An additional related protein, MBL/Ficolin Associated Protein (MAP- 1), which is present in low serum levels compared to MASP-1 and MASP-3, has been reported to function as a local lectin pathway specific complement inhibitor. Skjodt et al., Molecular Immunology, 47:2229-30 (2010). Accordingly MAP-1 itself, or fragments of
15 MAP-1, may be useful in the present invention as an inhibitor of MASP, and accordingly, as a lectin-pathway- specific inhibitor of complement activation. Finally, the ficolin family of proteins are characterized by carbohydrate binding and opsonic activities, sharing a structure similar to MBL. Like MBL, the ficolins have been shown to associate with MASPs in serum and may mediate complement activation in response to
20 pathogenic, necrotic, or apoptotic cell- specific carbohydrate markers. Accordingly, inhibitors of the ficolin family or functional fragments thereof may be useful in the present invention as an inhibitor of MASPs and as a lectin-pathway specific inhibitor of complement activation. US Patent 6,333,034 and US Patent 7,423,128; see also, WO 2008/154018 and WO 2009/110918.

25 In some embodiments, the inhibitor portion comprises an antibody (or antigen binding fragment thereof) that specifically binds to a human complement component protein (e.g., human C5, C6, C7, C8, or C9). The terms "specific binding" or "specifically binds" refer to two molecules forming a complex (e.g., a complex between an antibody and a complement component protein) that is relatively stable under
30 physiologic conditions. Typically, binding is considered specific when the association constant (K_a) is higher than 10^6 M^{-1} . Thus, an antibody can specifically bind to a C5

protein with a K_a of at least (or greater than) 10^6 (e.g., at least or greater than 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , 10^{14} , or 10^{15} or higher) M⁻¹. Examples of antibodies that specifically bind to a human complement component C5 protein are described in, e.g., U.S. patent no. 6,355,245 and PCT application publication no. WO 2010/015608.

5 Methods for determining whether an antibody binds to a protein antigen and/or the affinity for an antibody to a protein antigen are known in the art and described herein. For example, the binding of an antibody to a protein antigen can be detected and/or quantified using a variety of techniques such as, but not limited to, Western blot, dot blot, surface plasmon resonance method (e.g., BIAcore system; Pharmacia Biosensor
10 AB, Uppsala, Sweden and Piscataway, N.J.), or enzyme-linked immunosorbent assay (ELISA) assays. See, e.g., Harlow and Lane (1988) "Antibodies: A Laboratory Manual" Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Benny K. C. Lo (2004) "Antibody Engineering: Methods and Protocols," Humana Press (ISBN: 1588290921); Borrebaek (1992) "Antibody Engineering, A Practical Guide," W.H. Freeman and Co.,
15 NY; Borrebaek (1995) "Antibody Engineering," 2nd Edition, Oxford University Press, NY, Oxford; Johne et al. (1993) 1 Immunol Meth. 160: 191- 198; Jonsson et al. (1993) Ann Biol Clin 51: 19-26; and Jonsson et al. (1991) Biotechniques 11:620-627. See also, U.S. Patent No. 6,355,245.

20 In any of the embodiments described herein, the targeted molecule also includes an amino acid linker sequence linking the targeting portion and the inhibitor portion.

25 In some embodiments, a targeted molecule described herein comprises more than one (e.g., two, three, four, five, six, or seven or more) inhibitor portion e.g., more than one complement inhibitor polypeptide or drug described herein. The two or more inhibitor portions can be the same or different. For example, a targeted molecule described herein can comprise, in some embodiments, two or more soluble CD59 portions (e.g., soluble human CD59 portions). In another example, a targeted molecule described herein can contain two or more complement inhibitor polypeptide portions, wherein one is a soluble human CD59 and another is soluble human MCP. In another
30 example, a targeted molecule described herein can contain a complement inhibitor and a drug, e.g., one soluble CD59 portion and one corticosteroid. Thus, e.g., a targeted

molecule described herein can comprise: (a) a targeting portion (e.g., a C2 antibody, a B4 antibody, or an antigen-binding fragment of either of the foregoing); (b) a first inhibitor portion (e.g., a soluble form of CD59, e.g., human CD59); and (c) a second inhibitor portion (e.g., a soluble form of DAF, e.g., a soluble form of human DAF, or a corticosteroid such as prednisone). The inhibitor portion can be, e.g., any of those described herein including variants and biologically active fragments of the complement inhibitors described herein.

In some embodiments, the light chain of the targeting portion of the targeted molecule comprises at least one inhibitor portion and the heavy chain comprises at least one inhibitor portion. The two or more inhibitor portions can be the same or different. For example, in some embodiments, the targeted molecule comprises the Fab fragment of a targeting portion described herein, wherein: (i) the light chain of the Fab fragment comprises (at its C-terminal end) an inhibitor portion such as DAF, CD59, or any of the complement inhibitor polypeptides described herein and (ii) the heavy chain of the Fab fragment comprises (at its C-terminal end) the same or a different inhibitor portion as in (i), e.g., a complement inhibitor or a drug described herein. Appropriate pairing of the two chains can be expected to occur as an inherent property of the Fab. The inhibitor portion and the light chain or heavy chain of the Fab can be joined together directly or by way of a linker sequence (such as any of those described herein).

In one embodiment, the targeted molecule comprises B4Crry comprising the amino acid sequence of SEQ ID NO: 62. An exemplary nucleotide sequence encoding B4Crry is provided in SEQ ID NO: 61. In one embodiment, the targeted molecule comprises a homolog or biologically active fragment of B4Crry. Homologs of the B4Crry include proteins which differ from B4Crry described herein (or biologically-active fragment thereof) in that at least one or a few, but not limited to one or a few, amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide or fragment), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation, amidation and/or addition glycosylphosphatidyl inositol). For example, homologue of B4Crry may have an amino acid sequence that is at least about 70% identical to the amino acid sequence B4Crry (e.g., SEQ ID NO: 62), for example at least about any of 75%, 76%, 77%, 78%,

79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of B4Crry (e.g., SEQ ID NO: 62). Amino acid sequence identity can be determined in various ways, for example, using publicly available computer software such as BLAST, BLAST-2, ALIGN
5 or MEGALIGN™ (DNAST AR) software. One skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

In one embodiment, the targeted molecule comprises C2Crry comprising the amino acid sequence of SEQ ID NO: 64. An exemplary nucleotide sequence encoding
10 C2Crry is provided in SEQ ID NO: 63. In one embodiment, the targeted molecule comprises a homolog or biologically active fragment of C2Crry. Homologs of the C2Crry include proteins which differ from C2Crry described herein (or biologically-active fragment thereof) in that at least one or a few, but not limited to one or a few, amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide or
15 fragment), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation, amidation and/or addition glycosylphosphatidyl inositol). For example, homologue of C2Crry may have an amino acid sequence that is at least about 70% identical to the amino acid sequence C2Crry (e.g., SEQ ID NO: 64), for example at least about any of 75%, 76%, 77%, 78%,
20 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of C2Crry (e.g., SEQ ID NO: 64). Amino acid sequence identity can be determined in various ways, for example, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNAST AR) software. One skilled in the art can determine
25 appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

Preparation of Targeted Molecules

The molecules (described herein may be made by chemical synthesis
30 methods, or by linkage of a polynucleotide encoding the targeting portion (e.g. B4 or C2 antibody or fragment thereof) and a polynucleotide encoding the inhibitor portion (with

or without a linker sequence), and introducing the resulting polynucleotide molecule in a vector for transfecting host cells that are capable of expressing the molecule. Chemical synthesis, especially solid phase synthesis, is preferred for short peptides or those containing unnatural or unusual amino acids such as D-Tyr, Ornithine, and the like.

- 5 Recombinant procedures are preferred for longer polypeptides. The molecule can be isolated in vitro by protein purification methods. The molecule can also be provided "in situ" by introduction of a gene therapy system to the tissue of interest which then expresses the fusion.

Recombinant DNA techniques for making a fusion protein involves, in
10 simplified form, taking the a fusion protein-encoding polynucleotide, inserting it into an appropriate vector, inserting the vector into an appropriate host cell, and recovering or isolating the fusion protein produced thereby.

Provided herein are polynucleotides that encode the molecule. Such polynucleotide may also be used for delivery and expression of molecule. For example,
15 in some embodiments, there is provided a polynucleotide encoding a fusion protein comprising a targeting portion comprising an antibody or a fragment thereof described herein, and an inhibitor portion comprising an intact inhibitor molecule or a fragment thereof. In some embodiments, the polynucleotide also comprises a sequence encoding a signal peptide operably linked at the 5' end of the sequence encoding the fusion protein.
20 In some embodiments, a linker sequence is used for linking the targeting portion and the inhibitor portion

Also provided are expression vectors comprising a polynucleotide described herein for expression of the fusion protein. The expression vector can be used to direct expression of a fusion protein in vitro or in vivo. The vector may include any
25 element to establish a conventional function of a vector, for example, promoter, terminator, selection marker, and origin of replication. The promoter can be constitutive or regulative, and is selected from, for example, promoters of genes for galactokinase (GAL1), uridylyltransferase (GALT), epimerase (GAL10), phosphoglycerate kinase (PGK), glyceraldehydes-3-phosphate dehydrogenase (GPD), alcohol dehydrogenase
30 (ADH), and the like.

Many expression vectors are known to those of skill in the art. For example, *E. coli* may be transformed using pBR322, a plasmid derived from an *E. coli* species (Mandel et al., *J. Mol. Biol.*, 53:154 (1970)). Plasmid pBR322 contains genes for ampicillin and tetracycline resistance, and thus provides easy means for selection. Other
5 vectors include different features such as different promoters, which are often important in expression. For example, plasmids pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden), pKK233-2 (Clontech, Palo Alto, Calif., USA), and pGEM1 (Promega Biotech, Madison, Wis., USA), are all commercially available. Other vectors that can be used in the present invention include, but are not limited to, pET21a (Studier et al., *Methods*
10 *Enzymol.*, 185: 60-89 (1990)), pR1T5, and pR1T2T (Pharmacia Biotechnology), and pB0475 (Cunningham et al., *Science*, 243: 1330-1336 (1989); U.S. Pat. No. 5,580,723). Mammalian expression vectors may contain non-transcribed elements such as an origin of replication, promoter and enhancer, and 5' or 3' nontranslated sequences such as ribosome binding sites, a polyadenylation site, acceptor site and splice donor, and
15 transcriptional termination sequences. Promoters for use in mammalian expression vectors usually are for example viral promoters such as Polyoma, Adenovirus, HTLV, Simian Virus 40 (SV 40), and human cytomegalovirus (CMV). Vectors can also be constructed using standard techniques by combining the relevant traits of the vectors described above.

20 Also provided are host cells (such as isolated cells, transient cell lines, and stable cell lines) for expressing a targeted molecule described herein. The host cell may be prokaryotic or eukaryotes. Exemplary prokaryote host cells include *E. coli* K12 strain 294 (ATCC No. 31446), *E. coli* B, *E. coli* X1776 (ATCC No. 31537), *E. coli* W3110 (F-, gamma-, prototrophic/ATCC No. 27325), bacilli such as *Bacillus subtilis*, and other
25 enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcesans*, and various *Pseudomonas* species. One suitable prokaryotic host cell is *E. coli* BL21 (Stratagene), which is deficient in the OmpT and Lon proteases, which may interfere with isolation of intact recombinant proteins, and useful with T7 promoter-driven vectors, such as the pET vectors. Another suitable prokaryote is *E. coli* W3110 (ATCC No. 27325). When
30 expressed by prokaryotes the peptides typically contain an N-terminal methionine or a formyl methionine and are not glycosylated. In the case of fusion proteins, the N-terminal

methionine or formyl methionine resides on the amino terminus of the fusion protein or the signal sequence of the fusion protein. These examples are, of course, intended to be illustrative rather than limiting.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi
 5 or yeast are suitable cloning or expression hosts for fusion-protein-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, *Nature*, 290: 140 (1981); EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Pat. No. 4,943,529; Flier et al., *Bio/Technology*, 9:968-975 (1991)) such as, e.g., *K. lactis* (MW98-8C,
 10 CBS683, CBS4574; Louvencourt et al., *J. Bacteriol.*, 154(2):737-742 (1983)), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC No. 16,045), *K. wickerhamii* (ATCC No. 24,178), *K. waltii* (ATCC No. 56,500), *K. drosophilum* (ATCC No. 36,906; Van den Berg et al., *Bio/Technology*, 8:135 (1990)), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna et al., *J. Basic Microbiol.*, 28:265-278
 15 (1988)); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case et al., *Proc. Natl. Acad. Sci. USA*, 76:5259-5263 (1979)); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 Oct. 1990); and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 Jan. 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance et al., *Biochem. Biophys. Res. Commun.*,
 20 112:284-289 (1983); Tilburn et al., *Gene*, 26:205-221 (1983); Yelton et al., *Proc. Natl. Acad. Sci. USA*, 81: 1470-1474 (1984)) and *A. niger* (Kelly and Hynes, *EMBO J.*, 4:475-479 (1985)). Methylotropic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of
 25 specific species that are exemplary of this class of yeasts may be found in C. Anthony, *The Biochemistry of Methylotrophs*, 269 (1982). Host cells also include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9, as well as plant cells.

Examples of useful mammalian host cell lines include, but are not limited to, HeLa, Chinese hamster ovary (CHO), COS-7, L cells, C127, 3T3, BHK, CHL-1,
 30 NSO, HEK293, WI38, BHK, C127 or MDCK cell lines. Another exemplary mammalian cell line is CHL-1. When CHL-1 is used hygromycin is included as a eukaryotic selection

marker. CHL-1 cells are derived from RPMI 7032 melanoma cells, a readily available human cell line. Cells suitable for use in this invention are commercially available from the ATCC.

5 In some embodiments, the host cell is a non-human host cell. In some embodiment, the host cell is a CHO cell. In some embodiments, the host cell is a 293 cell.

The molecules can be isolated by a variety of methods known in the art. In some embodiments, when the targeted molecule is a fusion protein secreted into the growth media, the molecule can be purified directly from the media. If the fusion protein
10 is not secreted, it is isolated from cell lysates. Cell disruption can be done by any conventional method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. The molecules can be obtained by various methods. These include, but are not limited to, immunoaffinity chromatography, reverse phase chromatography, cation exchange chromatography, anion exchange chromatography,
15 hydrophobic interaction chromatography, gel filtration chromatography, and HPLC. For example, the molecule can be purified by immunoaffinity chromatography using an antibody that recognizes the targeting portion or an antibody that recognizes the inhibitor portion, or both. In some embodiments, the molecule is purified by ion change chromatography.

20 The peptide may or may not be properly folded when expressed as a fusion protein. These factors determine whether the fusion protein must be denatured and refolded, and if so, whether these procedures are employed before or after cleavage. When denaturing and refolding are needed, typically the peptide is treated with a chaotrope, such as guanidine HCl, and is then treated with a redox buffer, containing, for
25 example, reduced and oxidized dithiothreitol or glutathione at the appropriate ratios, pH, and temperature, such that the peptide is refolded to its native structure.

The molecules described herein may also contain a tag (such as a cleavable tag) for purification. This tag can be fused to the C-terminus or N-terminus of the targeting portion or the inhibitor portion, and can be used to facilitate protein
30 purification.

In some embodiments, the molecule could be synthesized de novo in whole or in part, using chemical methods well known in the art. For example, the component amino acid sequences can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography followed by chemical linkage to form a desired polypeptide. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing.

The molecules can be assayed for their desired properties using in vitro or in vivo assays, for example surface plasmon resonance or in vitro zymosan complement assay.

Targeted molecules in combination with an Immunosuppressant Agent

In one aspect, the present invention relates to a composition comprising an immunosuppressant agent in combination with a targeted molecule described herein. In one embodiment, the composition comprises a sub-therapeutic amount of an immunosuppressant agent in combination with a targeted molecule. In one embodiment, the immunosuppressant agent is selected from a list comprising but not limited to cyclosporine A, azathioprine, corticosteroids including prednisone, and methylprednisolone, cyclophosphamide, FK506 and mTOR inhibitors including rapamycin, sirolimus, and everolimus.

In one embodiment, the immunosuppressant agent is cyclosporine A which is an immunosuppressant drug widely used in organ transplantation to prevent rejection. It reduces the activity of the immune system by interfering with the activity and growth of T cells.

In one embodiment, the immunosuppressant agent is azathioprine, which is an immunosuppressive drug used in organ transplantation and autoimmune diseases and belongs to the chemical class of purine analogues. Azathioprine is an imidazolyl derivative and prodrug of mercaptopurine. In vivo, the metabolites of mercaptopurine are incorporated into replicating DNA, halting replication, as well as blocking the pathway for purine synthesis. It thus most strongly affects proliferating cells, such as the T cells and B cells of the immune system.

In one embodiment, the immunosuppressant agent is prednisone which is a synthetic corticosteroid drug that is used to treat certain inflammatory, some autoimmune diseases.

5 In one embodiment, the immunosuppressant agent is methylprednisolone which is a synthetic glucocorticoid or corticosteroid drug. It is a variant of prednisolone, methylated at carbon 6 of the B ring.

In one embodiment, the immunosuppressant agent is cyclophosphamide which is an alkylating agent of the nitrogen mustard type (specifically, the oxazaphosphorine group). Cyclophosphamide is used to treat cancers, autoimmune disorders and AL amyloidosis. As a prodrug, it is converted by liver cytochrome P450 (CYP) enzymes to form the metabolite 4-hydroxycyclophosphamide that has chemotherapeutic activity.

10 In one embodiment, the immunosuppressant agent is FK506 is an immunosuppressive drug used mainly after allogeneic organ transplant to lower the risk of organ rejection. It achieves this by inhibiting the production of interleukin-2, a molecule that promotes the development and proliferation of T cells.

In one embodiment, the immunosuppressant agent is an inhibitor of mTOR selected from a group comprising rapamycin, sirolimus, and everolimus.

20 Methods

The present invention provides methods for enhancing survival of transplant tissue. In one embodiment, the method comprises administering a composition comprising a targeted molecule to a subject who is the recipient of a transplant tissue. In one embodiment, the method comprises administering a composition comprising a targeted molecule to a subject before the subject has received the transplant tissue. In one embodiment, the method comprises administering a composition comprising a targeted molecule to a subject after the subject has received the transplant tissue. In one embodiment, the method comprises administering a composition comprising a targeted molecule to transplant tissue ex vivo, prior to introducing the transplant tissue into a recipient subject. In one embodiment, the method comprises administering a composition

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comprising a targeted molecule to a donor subject, prior to harvesting of the transplant tissue.

In one embodiment, the method comprises administering a composition comprising a targeted molecule in combination with an immunosuppressant agent to a
5 subject or tissue. In one embodiment, the method comprises administering a composition comprising a targeted molecule in combination with a sub-therapeutic level of an immunosuppressant agent to a subject or tissue. In one embodiment, the immunosuppressant agent is selected from a list comprising but not limited to cyclosporine A, azathioprine, corticosteroids including prednisone, and
10 methylprednisolone, cyclophosphamide, FK506, and mTOR inhibitors including rapamycin, sirolimus, and everolimus.

In certain embodiments, the composition comprises an amount of the immunosuppressant agent that is less than the amount necessary when the immunosuppressant agent is administered alone. For example, in certain embodiments,
15 the amount or concentration of immunosuppressant agent, when administered in combination with a targeted complement inhibitor described herein, is about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the amount or concentration of the immunosuppressant agent that is efficacious when administered alone.

20 In one embodiment, the transplant graft is obtained and modified ex vivo, using the targeted molecule and/or immunosuppressant agent composition described herein. In one embodiment, the transplant graft is obtained from a recipient, treated with the composition, and is then placed in contact or in the vicinity of the recipient. In certain embodiments, the transplant graft is placed in a container comprising a suitable media
25 and a composition comprising the targeted molecule and/or immunosuppressant agent, or variant thereof. In certain embodiments, the transplant graft is perfused with a suitable media and a composition comprising targeted molecule and/or immunosuppressant agent, or variant thereof.

In certain embodiments, the composition comprising the targeted molecule
30 and/or sub-therapeutic immunosuppressant agent or variant thereof is administered to the transplant graft following removal or harvest of the graft from a donor. For example, in

certain embodiments, the composition is administered to the transplant graft less than 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, 12 hours, 6 hours, 5 hours, 4 hours, 3 hours, 2 hours, 1 hour, 45 minutes, 30 minutes, 15 minutes, 10 minutes, 5 minutes, or 1 minute following removal or harvest of the graft from a donor. In certain embodiments, the composition is administered to the transplant graft for more than 72 hours, 48 hours, 36 hours, 24 hours, 18 hours, 12 hours, 6 hours, 5 hours, 4 hours, 3 hours, 2 hours, 1 hour, 45 minutes, 30 minutes, 15 minutes, 10 minutes, 5 minutes, or 1 minute prior to transplantation of the transplant graft into the recipient.

10 Uses of Targeted Molecules and Compositions Thereof

The targeted molecules described herein can function to specifically inhibit in vivo complement activation in the complement pathway and inflammatory manifestations that accompany it, such as recruitment and activation of macrophages, neutrophils, platelets, and mast cells, edema, tissue damage, and direct activation of local and endogenous cells. Compositions comprising these molecules can therefore be used for treatment of diseases or conditions that are mediated by excessive or uncontrolled activation of the complement system, particularly diseases or conditions mediated by excessive or uncontrolled activation of complement signaling. In some embodiments, there are provided methods of treating diseases involving local inflammation process.

In some embodiments, there is provided a method of treating a disease in which complement signaling is implicated in an individual, comprising administering to the individual an effective amount of a composition comprising a targeted molecule comprising: a) a targeting portion comprising an antibody or a fragment thereof, and b) an inhibitor portion comprising an inhibitor (for example a complement inhibitor) or a fragment thereof. In some embodiments, there is provided a method of inhibiting complement activation in an individual having a disease associated with complement activation, comprising administering to the individual an effective amount of a composition comprising a targeted molecule comprising: a) a targeting portion comprising an antibody or a fragment thereof, and b) an inhibitor portion comprising an inhibitor molecule or a fragment thereof. In some embodiments, there is provided a method of inhibiting inflammation in an individual having a disease associated with

complement activation, comprising administering to the individual an effective amount of a composition comprising a targeted molecule comprising: a) a targeting portion comprising an antibody or a fragment thereof, and b) an inhibitor portion comprising an inhibitor or a fragment thereof.

5 In some embodiments, the disease to be treated is ischemia reperfusion. Ischemia reperfusion (I/R) injury refers to inflammatory injury to the endothelium and underlying parenchymal tissues following reperfusion of hypoxic tissues. Ischemia reperfusion injury can result in necrosis and irreversible cell injury. The complement pathway (including the alternative complement pathway) is a major mediator of I/R
10 injury. Methods provided herein are thus useful for treatment of ischemia reperfusion that occurs in any organ or tissues, such as ischemia-reperfusion injury of any transplanted organ or tissue. Other conditions and diseases in which ischemia-reperfusion injury occurs will be known to those of skill in the art.

 In some embodiments, the present invention provides, methods of treating
15 and/or preventing graft rejection with provided compositions. In some embodiments, graft rejection may be autograft rejection, xenograft rejection, and/or allograft rejection. In some embodiments, the present invention provides methods of treating and/or preventing graft-versus-host disease.

20 Organ transplantation

 For dysfunctional, diseased, and/or otherwise undesired tissues or organs of the body, besides therapeutic intervention with drugs, organ and/or tissue transplantation is an alternative, and, in some cases, the last resort in the treatment of a patient. Particularly for patients with leukemia, end-stage renal, cardiac, pulmonary or
25 hepatic failure, organ transplantation is quite commonly used in the treatment.

 In some embodiments, allografts (organ grafts harvested from donors other than the patient him/herself or host/recipient of the graft) of various types, e.g. kidney, heart, lung, liver, bone marrow, pancreas, cornea, small intestine and skin (e.g. epidermal sheets) may be used. In some embodiments, xenografts (organ grafts harvested
30 from non-human animals), such as porcine heart valves, may be used to replace their dysfunctional human counterparts.

As an example, in some embodiments, tissue transplantation is or comprises bone marrow and/or stem cell transplantation. Bone marrow and/or stem cell transplantation has applications in a wide variety of clinical settings, including solid organ transplantation. A major goal in solid organ transplantation is the engraftment of the donor organ without a graft rejection immune response generated by the recipient, while preserving the immune-competence of the recipient against other foreign antigens. Typically, to prevent an undesired immune response, nonspecific immunosuppressive agents such as cyclosporin A, azathioprine, corticosteroids including prednisone, and methylprednisolone, cyclophosphamide, and FK506 are used. However, these agents must typically be administered on a daily basis and if stopped, graft rejection usually results. However, nonspecific immunosuppressive agents function by suppressing all aspects of the immune response, thereby greatly increasing a recipient's susceptibility to infections and diseases, including cancer. Accordingly, using the herein defined composition comprising a complement-targeted inhibitor in combination with a sub-therapeutic dose of an immunotherapeutic agent, and more safe and efficacious treatment plan is made herein available.

As another example, in some embodiments, tissue transplantation is or comprises hematopoietic tissue transplantation (e.g. bone marrow transplantation). In many embodiments, a goal of hematopoietic tissue transplantation is to achieve the successful engraftment of donor cells within a recipient host, such that immune and/or hematopoietic chimerism results. Chimerism is the reconstitution of the various compartments of the recipient's hematoimmune system with donor cell populations bearing MHC molecules derived from both, the allogeneic or xenogeneic donor, and a cell population derived from the recipient or, alternatively, the recipient's hematoimmune system compartments which can be reconstituted with a cell population bearing MHC molecules derived from only the allogeneic or xenogeneic marrow donor. Chimerism may vary from 100% (total replacement by allogeneic or xenogeneic cells) to low levels detectable only by molecular methods. Chimerism levels may vary over time and be permanent or temporary.

Autograft Rejection

In some embodiments, graft rejection refers to an autograft rejection, wherein the donor individual and recipient individual are the same (i.e., a patient's own tissue).

The present invention provides, among other things, methods of
5 administering to a recipient organism who has received or will receive a transplant of one or more heterologous tissue components from a donor organism, a composition comprising encapsulated recipient organism tissue components.

In some embodiments, one or more recipient tissue components are encapsulated within a nanoparticle. In some embodiments, one or more recipient tissue
10 components are encapsulated within a microbial cell. In some embodiments, one or more provided compositions are administered prior to the transplant. In some embodiments, one or more provided compositions are administered subsequent to the transplant.

Xenograft Rejection

15 In some embodiments, graft rejection refers to a xenograft rejection, wherein the donor and recipient are of different species. Typically, xenograft rejection occurs when the donor species tissue carries a xenoantigen against which the recipient species immune system mounts a rejection response.

The present invention provides, among other things, methods of
20 administering to a recipient organism who has received or will receive a transplant of one or more heterologous tissue components from a donor organism of different species a composition comprising encapsulated donor organism tissue components.

For example, in preparation for a heart valve transplant (e.g., from pig to human), donor porcine tissue components are isolated, encapsulated within nanoparticles,
25 and administered to a recipient organism.

Allograft Rejection

In some embodiments, graft rejection refers to an allograft rejection, wherein the donor individual and recipient individual are of the same species. Typically,
30 allograft rejection occurs when the donor tissue carries an alloantigen against which the recipient immune system mounts a rejection response.

The present invention provides, among other things, methods of administering to a recipient organism who has received or will receive a transplant of one or more heterologous tissue components from a donor organism a composition comprising encapsulated donor organism tissue components.

5 In some embodiments, one or more heterologous tissue components are encapsulated within a nanoparticle. In some embodiments, one or more heterologous tissue components are encapsulated within a microbial cell. In some embodiments, one or more heterologous tissue components are from a different species. In some embodiments, one or more provided compositions are administered prior to the transplant. In some
10 embodiments, one or more provided compositions are administered subsequent to the transplant.

In some embodiments, provided formulations may be used to treat patients with various forms of GvHD including acute and chronic GvHD that is either naive or refractory to conventional immunosuppressive agents such as steroids and cyclosporine
15 A. In some embodiments, provided formulations may be used as prophylaxis to prevent onset of GvHD by pretreating (i.e., tolerizing) the transplant recipient (i.e. host) prior to the transplantation and/or treating the recipient (i.e., host) within a certain time window post transplantation. In some embodiments, provided formulations may be used to treat patients with various forms of GvHD.

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Administration

In some embodiments, the host (e.g., transplant recipient) is pretreated with one or more compositions described herein.

In some embodiments, the host is treated with one or more compositions
25 comprises herein after receiving one or more tissue transplantation (i.e., tissue graft, organ transplant, etc.). Without wishing to be held to a particular theory, it is expected that administration of host tissue to the host pre- and/or post-transplantation will decrease the host immune response to the transplanted tissue.

The compositions described herein can be administered to an individual
30 via any route, including, but not limited to, intravenous (e.g., by infusion pumps), intraperitoneal, intraocular, intra-arterial, intrapulmonary, oral, inhalation, intravesicular,

intramuscular, intra-tracheal, subcutaneous, intrathecal, transdermal, transpleural, topical, inhalational (e.g., as mists or sprays), mucosal (such as via nasal mucosa), gastrointestinal, intraarticular, intracisternal, intraventricular, rectal (i.e., via suppository), vaginal (i.e., via pessary), intracranial, intraurethral, intrahepatic, and intratumoral. In some embodiments, the compositions are administered systemically (for example by intravenous injection). In some embodiments, the compositions are administered locally (for example by intraarterial or intraocular injection). In some embodiments, the compositions are administered by ex vivo incubation or perfusion.

10 Combination Therapy

In some embodiments, provided pharmaceutical formulations are administered to a subject in combination with one or more other therapeutic agents or modalities, for example, useful in the treatment of one or more diseases, disorders, or conditions treated by the relevant provided pharmaceutical formulation, so the subject is simultaneously exposed to both. In some embodiments, a provided tissue component composition is utilized in a pharmaceutical formulation that is separate from and distinct from the pharmaceutical formulation containing the other therapeutic agent. In some embodiments, a provided tissue component composition is admixed with the composition comprising the other therapeutic agent. In other words, in some embodiments, a provided tissue component composition is produced individually, and the provided tissue component composition is simply mixed with another composition comprising another therapeutic agent.

The particular combination of therapies (substances and/or procedures) to employ in a combination regimen will take into account compatibility of the desired substances and/or procedures and the desired therapeutic effect to be achieved. In some embodiments, provided formulations can be administered concurrently with, prior to, or subsequent to, one or more other therapeutic agents (e.g., desired known immunosuppressive therapeutics).

It will be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, a provided tissue component composition useful for treating transplant rejection may be administered concurrently with a

known immunosuppressant therapeutic that is also useful for treating transplant rejection), or they may achieve different effects (for example, a provided tissue component composition that is useful for treating transplant rejection may be administered concurrently with a therapeutic agent that is useful for alleviating adverse side effects, for instance, inflammation, nausea, etc.). In some embodiments, provided
5 tissue component compositions in accordance with the invention are administered with a second therapeutic agent.

As used herein, the terms "in combination with" and "in conjunction with" mean that the provided targeted molecule formulation can be administered concurrently
10 with, prior to, or subsequent to, one or more other desired therapeutics such as an immunosuppressant agent including but not limited to a sub-therapeutic dose of such an immunosuppressant agent. In general, each substance will be administered at a dose and/or on a time schedule determined for that agent.

In certain embodiments, the method comprises administering a
15 composition comprising a combination of an immunosuppressive agent and a targeted inhibitor described herein.

In certain embodiments, the method comprises administering one or more compositions. For example, in one embodiment, the method comprises administering a first composition comprising an immunosuppressive agent and a second composition
20 comprising a targeted inhibitor described herein. The different compositions may be administered to the subject in any order and in any suitable interval. For example, in certain embodiments, the one or more compositions are administered simultaneously or near simultaneously. In certain embodiments, the method comprises a staggered administration of the one or more compositions, where a first composition is
25 administered and a second composition administered at some later time point. Any suitable interval of administration which produces the desired therapeutic effect may be used.

In certain embodiments, the method has an additive effect, wherein the overall effect of the administering a combination of therapeutic agents or procedures is
30 approximately equal to the sum of the effects of administering each therapeutic agent or procedure alone. In other embodiments, the method has a synergistic effect, wherein the

overall effect of administering a combination of therapeutic agents or procedures is greater than the sum of the effects of administering each therapeutic agent or procedure alone.

5 Treatments and Therapies

In some embodiments, to prevent graft rejection, the host receives medications, chemotherapy (i.e., destroy host bone marrow), total body irradiation, and other antibody medications before receiving donor tissue transplant. In some embodiments, chance of graft rejection is related to the match between the donor and host MHC antigens, the overall genetic relationship between donor and host, and the type of disease for which the transplantation has been performed.

In some embodiments, immunosuppressive therapy includes administration of corticosteroids (e.g., prednisolone, hydrocortisone, etc.), calcineurin inhibitors (e.g., cyclosporine, tacrolimus, etc.), anti-proliferatives (e.g., azathioprine, mycophenolic acid, etc.), mTOR inhibitors (e.g., sirolimus, rapamycin, everolimus, etc.), and/or combinations thereof. In some embodiments, a short course of high-dose corticosteroids is applied and repeated. In some embodiments, corticosteroids are co-administered with one or more calcineurin inhibitors and one or more anti-proliferative agents. In some embodiments mTOR inhibitors are used where calcineurin inhibitors or steroids are contraindicated.

In some embodiments, antibody-based therapy includes administration of one or more antibodies or antibody-based drugs specific to select immune system components (e.g., IL-2R. α . receptor, CD20, T-cells, etc.). Non-limiting examples of antibodies or antibody-based drugs include monoclonal anti-IL-2R. α . receptor antibodies (e.g., Basiliximab, Daclizumab, etc.), polyclonal anti-T-cell antibodies (e.g., anti-thymocyte globulin [ATG], anti-lymphocyte globulin [ALG], etc.), monoclonal anti-T-cell antibodies (e.g., muromonab-CD3, Orthoclone OKT3, etc.), and monoclonal anti-CD20 antibodies (e.g., Rituximab).

30 Graft versus Host Disease (GvHD)

Graft-versus-host disease (GvHD) is a common complication following an allogeneic or xenogenic tissue transplant (e.g. hematopoietic cell transplant, tissue implantation, graft, etc.). GvHD occurs when transplanted donor immune cells (e.g., white blood cells including T cells) present in the graft (i.e. donor) tissue recognize the host (i.e., recipient) as "non-self" (i.e., antigenically "foreign") and attack the tissues of the recipient. Donor infection-fighting cells attack tissues in the host just as if they were attacking an infection. For example, in some embodiments, bone marrow transplant presents risk of GvHD, because mature donor lymphocytes present within transplanted donor marrow may recognize recipient tissues as foreign. The transplanted donor immune cells then attack the host's body cells and destroy them. GvHD is most commonly associated with hematopoietic stem cell or bone marrow transplant, but applies to other forms of tissue graft as well.

In some embodiments, three criteria must be met in order for GvHD to occur. One, an immune-competent graft (i.e., donor tissue) is administered (i.e., transplanted to host), with viable and functional donor immune cells. Two, the host is immunologically disparate (i.e., histo-incompatible). Three, the host is immune-compromised and cannot destroy or inactivate the transplanted donor immune cells.

After bone marrow transplantation, lymphocytes (i.e., T cells) present in the graft attack the tissues of the transplant host after perceiving host tissues as antigenically foreign. The donor lymphocytes produce an excess of cytokines, including TNF-alpha (TNF- α) and interferon-gamma (IFN γ).

A wide range of host antigens can initiate GvHD, among them human MHCs. However, GvHD can occur even when MHC-identical siblings are donors. MHC-identical siblings or MHC-identical unrelated donors often have genetically different proteins (i.e., minor histocompatibility antigens) that can be presented by MHC molecules to the donor's T-cells, which see these antigens as foreign and so mount an immune response.

While donor T-cells are undesirable as effector cells of GvHD, they are valuable for engraftment by preventing the host residual immune system from rejecting the bone marrow graft (i.e., host-versus-graft; discussed above), preventing the host from infection, and more importantly mediating graft-versus-tumor effect.

In some embodiments, GvHD is either acute GvHD (aGVHD) or chronic GvHD (cGVHD). Acute GvHD (aGVHD) usually occurs within the first three months following a transplant, and can affect the skin, liver, lung, stomach, and/or intestines.

5 In some embodiments, symptoms of aGVHD include rash, yellow skin and eyes due to elevated concentrations of bilirubin, and diarrhea. Acute GvHD is graded on a scale of 1 to 4; grade 4 is the most severe. In some embodiments, aGVHD can be fatal.

Chronic GvHD (cGVHD) is the late form of the disease, and usually develops three months or more after a transplant. The symptoms of cGVHD resemble spontaneously occurring autoimmune disorders such as lupus or scleroderma.

10 In some embodiments, GvHD is more easily prevented than treated. Preventive measures include the administration of cyclosporin with or without methotrexate or steroids after stem cell transplant. In some embodiments T lymphocytes are removed from the hematopoietic stem cell graft before it is transplanted.

In some embodiments, first-line treatment of GvHD includes steroid
15 therapy. In some embodiments, chronic GvHD occurs approximately in 10-40 percent of patients after hematopoietic stem cell transplant. Symptoms vary more widely than those of acute GvHD and are similar to various autoimmune disorders. In some embodiments, symptoms include dry eyes, dry mouth, rash, ulcers of the skin and mouth, joint contractures (i.e., inability to move joints easily), abnormal blood test results of liver
20 function, stiffening of the lungs (i.e., difficulty in breathing), inflammation in the eyes, difficulty in swallowing, muscle weakness, a white film in the mouth, and/or combinations thereof. In some embodiments, the incidence of GvHD increases with increasing degree of mismatch between donor and host HLA antigens, increasing donor age and increasing host age.

25

Pharmaceutical Compositions

Also provided herein are pharmaceutical compositions comprising the targeted molecule and/or immunosuppressant and a pharmaceutically acceptable carrier. The pharmaceutical compositions may be suitable for a variety of modes of
30 administration described herein, including for example systemic or localized administration. The pharmaceutical compositions can be in the form of eye drops,

injectable solutions, or in a form suitable for inhalation (either through the mouth or the nose) or oral administration. The pharmaceutical compositions described herein can be packaged in single unit dosages or in multidosage forms.

In some embodiments, the pharmaceutical compositions comprise a
5 pharmaceutically acceptable carrier suitable for administration to human. In some
embodiments, the pharmaceutical compositions comprise a pharmaceutically acceptable
carrier suitable for intraocular injection. In some embodiments, the pharmaceutical
compositions comprise a pharmaceutically acceptable carrier suitable for topical
application. In some embodiments, the pharmaceutical compositions comprise a
10 pharmaceutically acceptable carrier suitable for intravenous injection. In some
embodiments, the pharmaceutical compositions comprise and a pharmaceutically
acceptable carrier suitable for injection into the arteries.

The compositions are generally formulated as sterile, substantially
isotonic, and in full compliance with all Good Manufacturing Practice (GMP) regulations
15 of the U.S. Food and Drug Administration. In some embodiments, the composition is free
of pathogen. For injection, the pharmaceutical composition can be in the form of liquid
solutions, for example in physiologically compatible buffers such as Hank's solution or
Ringer's solution. In addition, the pharmaceutical composition can be in a solid form and
redissolved or suspended immediately prior to use. Lyophilized compositions are also
20 included.

For oral administration, the pharmaceutical compositions can take the
form of, for example, tablets or capsules prepared by conventional means with
pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinized maize
starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose,
25 microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium
stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or
wetting agents (e.g., sodium lauryl sulfate). Liquid preparations for oral administration
can take the form of, for example, solutions, syrups or suspensions, or they can be
presented as a dry product for constitution with water or other suitable vehicle before use.
30 Such liquid preparations can be prepared by conventional means with pharmaceutically
acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives

or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., ationd oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

The present invention in some embodiments provides compositions comprising a targeted molecule and a pharmaceutically acceptable carrier suitable for administration to the eye. Such pharmaceutical carriers can be sterile liquids, such as water and oil, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, and the like. Saline solutions and aqueous dextrose, polyethylene glycol (PEG) and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, sodium state, glycerol monostearate, glycerol, propylene, water, and the like. The pharmaceutical composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The targeted molecule and other components of the composition may be encased in polymers or fibrin glues to provide controlled release of the molecule. These compositions can take the form of solutions, suspensions, emulsions, ointment, gel, or other solid or semisolid compositions, and the like. The compositions typically have a pH in the range of 4.5 to 8.0. The compositions must also be formulated to have osmotic values that are compatible with the aqueous humor of the eye and ophthalmic tissues. Such osmotic values will generally be in the range of from about 200 to about 400 milliosmoles per kilogram of water ("mOsm/kg"), but will preferably be about 300 mOsm/kg.

In some embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for injection intravenously, intraperitoneally, or intravitreally. Typically, compositions for injection are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of

active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compositions may further comprise additional ingredients, for example preservatives, buffers, tonicity agents, antioxidants and stabilizers, nonionic wetting or clarifying agents, viscosity-increasing agents, and the like.

Suitable preservatives for use in a solution include polyquaternium-1, benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, benzethonium chloride, and the like. Typically (but not necessarily), such preservatives are employed at a level of from 0.001% to 1.0% by weight.

Suitable buffers include boric acid, sodium and potassium bicarbonate, sodium and potassium borates, sodium and potassium carbonate, sodium acetate, sodium biphosphate and the like, in amounts sufficient to maintain the pH at between about pH 6 and pH 8, and preferably, between about pH 7 and pH 7.5.

Suitable tonicity agents are dextran 40, dextran 70, dextrose, glycerin, potassium chloride, propylene glycol, sodium chloride, and the like, such that the sodium chloride equivalent of the ophthalmic solution is in the range 0.9 plus or minus 0.2%.

Suitable antioxidants and stabilizers include sodium bisulfite, sodium metabisulfite, sodium thiosulfite, thiourea and the like. Suitable wetting and clarifying agents include polysorbate 80, polysorbate 20, poloxamer 282 and tyloxapol. Suitable viscosity-increasing agents include dextran 40, dextran 70, gelatin, glycerin, hydroxyethylcellulose, hydroxymethylpropylcellulose, lanolin, methylcellulose, petrolatum, polyethylene glycol, polyvinyl alcohol, polyvinylpyrrolidone, carboxymethylcellulose and the like.

The use of viscosity enhancing agents to provide topical compositions with viscosities greater than the viscosity of simple aqueous solutions may be desirable. Such viscosity building agents include, for example, polyvinyl alcohol, polyvinyl pyrrolidone, methyl cellulose, hydroxy propyl methylcellulose, hydroxyethyl cellulose,

carboxymethyl cellulose, hydroxy propyl cellulose or other agents known to those skilled in the art. Such agents are typically employed at a level of from 0.01% to 2% by weight.

In some embodiments, there is provided a pharmaceutical composition for delivery of a nucleotide encoding the targeted molecule, described herein. The pharmaceutical composition for gene therapy can be in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle or compound is imbedded. Alternatively, where the complete gene delivery system can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical composition can comprise one or more cells which produce the gene delivery system.

In clinical settings, a gene delivery system for a gene therapeutic can be introduced into a subject by any of a number of methods. For instance, a pharmaceutical composition of the gene delivery system can be introduced systemically, e.g., by intravenous injection, and specific transduction of the protein in the target cells occurs predominantly from specificity of transfection provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited with introduction into the animal being quite localized. For example, the gene delivery vehicle can be introduced by catheter, See U.S. Pat. No. 5,328,470, or by stereotactic injection, Chen et al. (1994), Proc. Natl. Acad. Sci., USA 91: 3054-3057. A polynucleotide encoding a targeted molecule can be delivered in a gene therapy construct by electroporation using techniques described, Dev et al. (1994), Cancer Treat. Rev. 20:105-115.

Dosing

The optimal effective amount of the compositions can be determined empirically and will depend on the type and severity of the disease, route of administration, disease progression and health, mass and body area of the individual. Such determinations are within the skill of one in the art. The effective amount can also be determined based on in vitro complement activation assays. Examples of dosages of molecules which can be used for methods described herein include, but are not limited to, an effective amount within the dosage range of any of about 0.01 mg/kg to about 300

mg/kg, or within about 0.1 mg/kg to about 40 mg/kg, or with about 1 mg/kg to about 20 mg/kg, or within about 1 mg/kg to about 10 mg/kg. In some embodiments, the amount of composition administered to an individual is about 10 mg to about 500 mg per dose, including for example any of about 10 mg to about 50 mg, about 50 mg to about 100 mg, about 100 mg to about 200 mg, about 200 mg to about 300 mg, about 300 mg to about 500 mg, about 500 mg to about 1 mg, about 1 mg to about 10 mg, about 10 mg to about 50 mg, about 50 mg to about 100 mg, about 100 mg to about 200 mg, about 200 mg to about 300 mg, about 300 mg to about 400 mg, or about 400 mg to about 500 mg per dose.

The compositions may be administered in a single daily dose, or the total daily dose may be administered in divided dosages of two, three, or four times daily. The compositions can also be administered less frequently than daily, for example, six times a week, five times a week, four times a week, three times a week, twice a week, once a week, once every two weeks, once every three weeks, once a month, once every two months, once every three months, or once every six months. The compositions may also be administered in a sustained release formulation, such as in an implant which gradually releases the composition for use over a period of time, and which allows for the composition to be administered less frequently, such as once a month, once every 2-6 months, once every year, or even a single administration. The sustained release devices (such as pellets, nanoparticles, microparticles, nanospheres, microspheres, and the like) may be administered by injection or surgical implantation in various locations.

Dosage amounts and frequency will vary according the particular formulation, the dosage form, and individual patient characteristics. Generally speaking, determining the dosage amount and frequency for a particular formulation, dosage form, and individual patient characteristic can be accomplished using conventional dosing studies, coupled with appropriate diagnostics.

Unit Dosages, Articles of Manufacture, and Kits

Also provided are unit dosage forms of compositions, each dosage containing from about 0.01 mg to about 50 mg, including for example any of about 0.1 mg to about 50 mg, about 1 mg to about 50 mg, about 5 mg to about 40 mg, about 10 mg

to about 20 mg, or about 15 mg of the targeted molecule. In some embodiments, the unit dosage forms of targeted molecule composition comprise about any of 0.01 mg-0.1 mg, 0.1 mg-0.2 mg, 0.2 mg-0.25 mg, 0.25 mg-0.3 mg, 0.3 mg-0.35 mg, 0.35 mg-0.4 mg, 0.4 mg-0.5 mg, 0.5 mg-1.0 mg, 10 mg-20 mg, 20 mg-50 mg, 50 mg-80 mg, 80 mg-100 mg, 100 mg-150 mg, 150 mg-200 mg, 200 mg-250 mg, 250 mg-300 mg, 300 mg-400 mg, or 400 mg-500 mg targeted molecule. In some embodiments, the unit dosage form comprises about 0.25 mg targeted molecule. The term "unit dosage form" refers to a physically discrete unit suitable as unitary dosages for an individual, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier, diluent, or excipient. These unit dosage forms can be stored in suitable packaging in single or multiple unit dosages and may also be further sterilized and sealed.

The present invention also provides kits comprising compositions (or unit dosages forms and/or articles of manufacture) described herein and may further comprise instruction(s) on methods of using the composition, such as uses described herein. The kits described herein may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein.

EXPERIMENTAL EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the present invention and practice the claimed methods. The following working examples therefore, specifically point out the preferred embodiments of the present

invention, and are not to be construed as limiting in any way the remainder of the disclosure.

Example 1: Targeted complement inhibition protects vascularized composite allografts from acute graft injury.

Experiments were conducted to evaluate the effects of antibody-targeted complement inhibition on acute graft injury. VC isografts were transplanted into Rag -/- mice or wildtype mice. Mice were treated with PBS, B4 IgM mAb or B4-Crry. Isolated VCAs were stained and scored using a histology scoring system. Ischemia reperfusion injury (IRI) VC isografts was observed in the PBS treated recipients (PBS Iso) as measured by both skin and muscle histopathological score (Figure 1). Protection from IRI was observed in Rag-/- mice that do not contain Abs (Rag Iso). Further, restoration of IRI was observed in Rag-/- mice when reconstituted with B4 IgM mAb. Finally, protection from IRI was observed in wild type mice treated with B4scFv-Crry (Iso B4-Crry (Figure 1).

Experiments are designed to evaluate whether antibody-targeted complement inhibition combined with a subtherapeutic dose of an immunosuppressant affects survival time. Animals are treated with either PBS-vehicle or B4-Crry and subtherapeutic cyclosporine A or FK506 (Tacrolimus). Survival after transplantation is measured.

Example 2: Inhibition of Alternative Complement pathway in Target Organs represents a novel and effective approach to control GVHD while sparing graft-versus-leukemia (GVL) Effect

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective immunotherapy for hematological malignancies. However, application of allo-HSCT is limited by GVHD, and separation of GVH and GVL response is a great challenge in the field. Complement can be activated via three different pathways: classical, lectin and alternative pathway. Owing to the auto-activation property and crucial in “amplification loop”, alternative complement pathway (ACP) plays a central

role in the development of autoimmune diseases such as colitis and inflammatory bowel disease, which share certain pathogenesis with intestinal GVHD.

C3d deposition at GVHD target organs

5 Complement activation has been shown to implicate in GVHD development in pre-clinical and clinical studies. However, how complement pathways regulate GVHD has not been elucidated. Complement decomposition was evident at GVHD target organs (Figure 2), suggesting local complement activation contributes to GVHD pathogenesis. To functionally dissect the role of complement pathways during
10 GVHD, mice deficient for fB or C1q/MLB were used as recipients in which the ACP or classical/actin pathway is altered. To increase translational potential, the effects of ACP on GVHD and GVL responses were evaluated using B4-Crry

Deficiency of fB, not C1q/MLB, in the host ameliorates GVHD

15 To functionally dissect the role of complement pathways during GVHD, mice deficient for fB or C1q/MLB were used as recipients in which the ACP or classical/actin pathway is altered. In MHC-mismatched murine models of GVHD, it was determined that GVHD severity and mortality was significantly reduced in fB but not C1q/MLB deficient recipients compared to wild type (WT) counterpart, suggesting a
20 crucial role of ACP in GVHD pathogenesis after allo-HSCT, as shown in Figure 3. Experiments are conducted to evaluate whether systematic treatment of recipients with B4-Crry ameliorates GVHD, as measured by longer survival and lower GVHD clinical score.

Mechanisms for GVHD attenuation caused by host alternative complement pathway deficiency

25 Mechanistic studies revealed that ACP deficiency in the host had a significant impact on phenotype of immune cells in GVHD target organs rather than in lymphoid organs. Complement decomposition was decreased at GVHD target organs of
30 ACP-deficient recipients. Gut integrity was significantly preserved in fB-/- than WT recipients (Figure 4A). The activation and maturation of host DCs in GVHD target

organs such as gut, lung, and thymus were significantly lower while GVHD-protective host CD8+DCs were higher in fB-/- recipients. On contrary, ACP deficiency did not affect the phenotype of recipient DCs in the lymphoid organs. The numbers of donor T cells and particularly CD103+CD8+ T cells, a cell subset critically mediating intestinal GVHD, were decreased in the gut of fB-/- recipients. Consistently, in the fB-/- recipients, donor T cells reduced the expression of gut homing chemokine CCR9. Donor Th1 and Tc1 cells were significantly decreased whereas donor iTregs were increased in the liver of fB-/- recipients (Figure 4B). Meanwhile, donor Th2 and Tc1 were diminished in the lung. The cytolytic activity of liver-infiltrated lymphocyte was also lower in the absence of host ACP. The thymic integrity was significantly improved in fB-/- recipients. No differences in differentiation and function of donor T cells in lymphoid organs were found in the fB-/- recipients (Figure 4C). These studies suggest that complement activation in target organs is important for GVHD pathogenesis.

Taken together, this study identifies a central role of ACP in GVHD and validates ACP as a therapeutic target for the control of GVHD. It provides evidences that locally-generated complement at GVHD target organs is important for GVHD development. This study proposes a novel strategy to spare GVHD and GVL effect. Because site-specific complement inhibition is expected to cause fewer side effects and human ACP inhibitor TT30 is currently available, the current finding has a high translational potential in clinic.

Example 3: In vitro characterization of MAp44-B4scFv

A construct was designed which includes MAp44 linked to a single chain antibody fragment of B4 (MAp44-B4scFv; SEQ ID NO: 60). A binding (targeting) assay is conducted where an ELISA plate coated with C3d or BSA is incubated with various concentrations of MAp44-B4scFv. The plates are then washed and binding is detected by means of anti-Map44 mAb. A lectin pathway inhibition assay is conducted using a lectin pathway-specific assay using mannan coated ELISA plates.

Example 4: B4scFv targeting strategy

The B4scFv targeting strategy was evaluated in a cardiac Tx model: By transplanting hearts from wt donor mice into Ab-deficient mice reconstituted with specific self-reactive IgM mAbs, neoepitopes expressed post-transplant were identified, and a key role for IgM recognition of these epitopes was demonstrated in graft injury. A therapeutic strategy that exploited this post-ischemia recognition system of natural antibodies was then developed and characterized. Based on neoepitope identification, an anti-annexin-IV single chain antibody (B4scFv) and an scFv linked to Crry (B4scFv-Crry) was constructed. In an allograft transplant model, in which recipients contain a full natural antibody repertoire, both constructs blocked graft IgM binding and complement activation, and significantly reduced cardiac graft inflammation and injury. Furthermore, B4scFv-Crry specifically targeted to the transplanted heart and, unlike complement deficiency, did not affect immunity to infection. Further, post-ischemic expression of the B4scFv neoepitope has been observed in multiple tissues/organs in both mouse and humans. Further, the complement inhibitory construct, B4scFv-Crry, was observed to directly bind to post-ischemic human liver and brain. Liver biopsies were obtained from human donor livers following Tx and 1 hour reperfusion. Sections stained positive for annexin IV expression using B4 mAb for immunohistochemistry, with a similar sinusoidal pattern of deposition to that seen in post-ischemic mouse liver sections; no staining was detected with control F632 (anti-NP) IgM mAb. Also, B4scFv-Crry stained biopsies obtained post-Tx, but not samples from normal biopsies. Similar data was obtained with normal and ischemic brain samples obtained from stroke patients. Finally, it is shown herein that a decrease in anti-annexin IV Abs in human serum 24 hours after cardiac Tx as compared to pre-Tx levels (note that levels were not decreased in 2 patients, which is likely due to transfusion volume) (Table 1). B4scFv is injury-specific and does not bind normal tissue. In certain aspects, there may be benefits of a neoepitope vs. C3d targeting approach for protection against IRI in a transplant. First, it will target the proximal event in complement activation, and does not depend on prior complement activation (unlike CR2). Second, the targeting vehicle itself contributes to therapeutic activity by blocking the binding of complement activating pathogenic IgM, which in turn reduces the binding of C1q and MBL, which can impact inflammation, endothelial activation, cell trafficking, and Ag-presentation. Third, it is likely less

immunosuppressive since not all sites of infection and C3 deposition will be targeted with complement inhibition. Fourth, it does not limit expression of its own ligand.

Patient	Pre-Transplant (O.D)	Post Transplant (24hrs)(O.D)
1	0.75	0.23
2	0.54	0.52
3	0.68	0.32
4	0.57	0.49
5	0.76	0.35
6	0.84	0.44

Table 1. Semi-quantitative ELISA for Annexin IV antibodies in human transplant patient serum. Serum levels of Annexin IV were measured pre and 24 hrs post transplantation. Note that patients 1, 3, 5, and 6 show a decrease in relative O.D. at 450nm at 24 hrs as compared to pre-transplant levels.

Further it is demonstrated herein that following perfusion of radiolabeled B4scFv-Crry through cardiac grafts of BD or living donors, there is a 7-fold higher level of B4scFv-Crry binding in BD vs. living donor grafts, further emphasizing the important role of BD in immune priming, and validating the B4 targeting strategy (Figure 5). In this experiment, B4scFv-Crry was administered to the donor hearts as a constituent of the UW preservation solution. A clinically relevant procurement protocol was used where following ligation of the superior and inferior vena cava in the thoracic cavity, stainless steel micro serrefines was used to clamp the thoracic aorta. The donor heart was then isolated and perfused with 0.5 mL ice cold UW or I¹²⁵-B4scFv-Crry via the proximal aortic arch.

Example 5: B4-Crry and subtherapeutic rapamycin therapy

Experiments are conducted utilizing an aortic interposition model of allograft vasculopathy (AV) to determine the impact of acute B4-Crry therapy on the development of AV. Current maintenance immunosuppressive therapies have been targeted to either specifically or globally dampening effector T cell responses many of which prevent the viability and proliferative capacity of tolerance inducing regulatory T

cells (Treg). On the other hand, it has been shown that rapamycin can foster the stability, expansion, and natural suppressive capacity of these Treg. Interestingly, both complement inhibition and rapamycin selectively allow for the proliferation of Treg while inhibiting the growth of effector T cells. Furthermore, two recent reports
5 demonstrate that rapamycin and complement inhibition, used individually, can bolster the stability and half-life of Tregs in nonhuman primates and rodent models of transplantation and autoimmunity, respectively. While rapamycin potent Treg inducing capacity is well documented it is seldom used in clinical Tx due to severe side-effects associated with poor wound healing, patient tolerability issues, infection and cancer. A
10 number of novel strategies have been employed in an attempt to utilize rapamycin, one such is the use of rapamycin at subtherapeutic doses combine with ex-vivo expanded Tregs to support therapeutic levels of Treg proliferation in vivo.

The strategy described herein employs a sub-therapeutic rapamycin dosing strategy, that does not alone protect against AV development, combined with early
15 complement inhibition with B4-Crry (C3 convertase inhibitor) and anti-C5 (C5 and MAC inhibitor) administered at 0 and 24 hrs post reperfusion. Six groups are utilized, 1. No treatment control, 2. Rapamycin administered at 300ng dose on days 7, 9, 10, and 11 post transplantation, 3. B4-Crry alone, 4. Anti-C5 alone, 5. B4-Crry + Rapamycin, and 6. Anti-C5 + Rapamycin.

20

Example 6: B4-Crry and subtherapeutic tacrolimus

Experiments were conducted to examine the effects of B4-Crry in combination with sub-therapeutic tacrolimus (also known as FK506). Tacrolimus was given at 1mg/kg/day. This is a subtherapeutic dose that does not prolong survival. Thus,
25 untreated allogeneic grafts reject at the same extent as the subtherapeutic Tacrolimus treated animals. B4-Crry was given at 0 and 24 hours post-transplant and Tacrolimus was given at 1mg/kg/day for the duration of the experiment (until full rejection of the graft).

The survival of the grafts was assessed over time, which demonstrates that acute B4-Crry treatment combined with subtherapeutic immunosuppression with
30 tacrolimus significantly prolongs graft survival, as compared to control tacrolimus alone.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by
5 others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed is:

1. A composition for treating or preventing an injury associated with transplantation, comprising (a) a targeted inhibitor molecule wherein the targeted inhibitor molecule comprises a targeting portion and an inhibitor portion, wherein the molecule inhibits complement pathways, and (b) a sub-therapeutic dose of an immunosuppressant agent.
2. A composition of claim 1 wherein the targeting portion comprises an antibody or fragment thereof that specifically binds to Annexin IV, a post-translational modification found on Annexin IV and other proteins, or a phospholipid.
3. A composition of claim 1 wherein the inhibitor portion comprises at least one selected from the group consisting of FH, MCP, DAF, Crry, MAp44, CD59, and CR1.
4. A composition of claim 1 wherein the immunosuppressant agent comprises an agent selected from the group consisting of cyclosporine A, azathioprine, a corticosteroid including prednisone, and methylprednisolone, cyclophosphamide, FK506, and an mTOR inhibitor selected from a group consisting of rapamycin, sirolimus, and everolimus.
5. A method of treating or preventing an injury associated with transplantation wherein complement is activated, the method comprising administering to a subject in need thereof a therapeutically effective amount of a therapeutic agent comprising a targeted inhibitor molecule comprising a targeting portion and an inhibitor portion, wherein the molecule inhibits complement activation.
6. A method of claim 5 wherein the transplant is a vascularized composite allograft, heart transplant, kidney transplant, liver transplant, lung transplant,

pancreas transplant, intestine transplant, thymus transplant, musculoskeletal graft, cornea graft, skin graft, heart valve graft, nerves graft or vein graft.

7. A method of claim 5 wherein the transplant is an allogeneic hematopoietic stem cell transplantation.

8. A method of claim 5 wherein the targeting portion comprises an antibody or fragment thereof that specifically binds to Annexin IV, a post-translational modification found on Annexin IV and other proteins, or a phospholipid.

9. A method of claim 5 wherein the inhibitor portion comprises at least one selected from the group consisting of FH, MCP, DAF, Crry, MAp44, CD59, and CR1.

10. The method of claim 5, further comprising administering to the subject a sub-therapeutic amount of an immunosuppressant agent.

11. A method of claim 10, wherein the immunosuppressant agent comprises an agent selected from the group consisting of cyclosporine A, azathioprine, a corticosteroid including prednisone, and methylprednisolone, cyclophosphamide, FK506, and an mTOR inhibitor selected from a group consisting of rapamycin, sirolimus, and everolimus.

12. The method of claim 5, wherein the subject has graft versus host disease.

13. The method of claim 5, wherein the subject has an ischemia reperfusion injury of the transplant or at risk for developing an ischemia reperfusion injury of the transplant.

14. A method of treating or preventing an injury associated with a transplant comprising administering to a transplant a therapeutically effective amount of

a therapeutic agent comprising a targeted inhibitor molecule comprising a targeting portion and an inhibitor portion, wherein the molecule inhibits complement pathways.

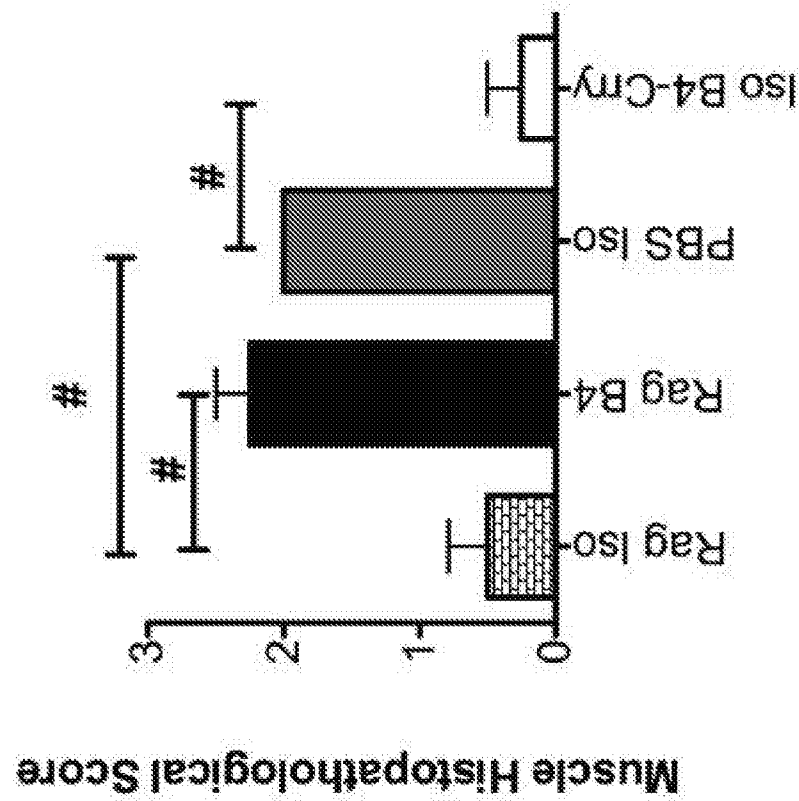
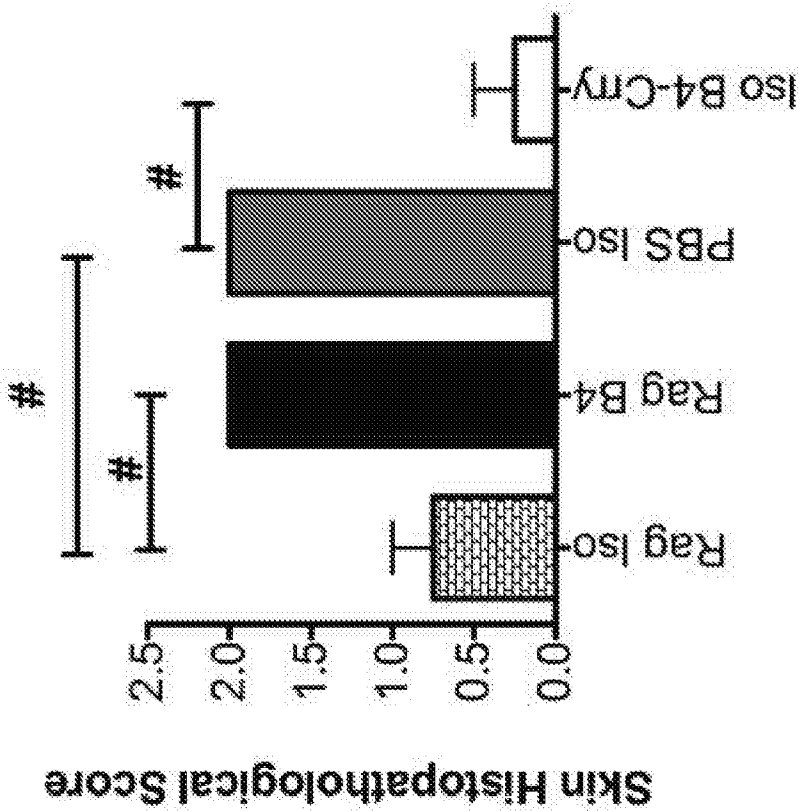


Figure 1

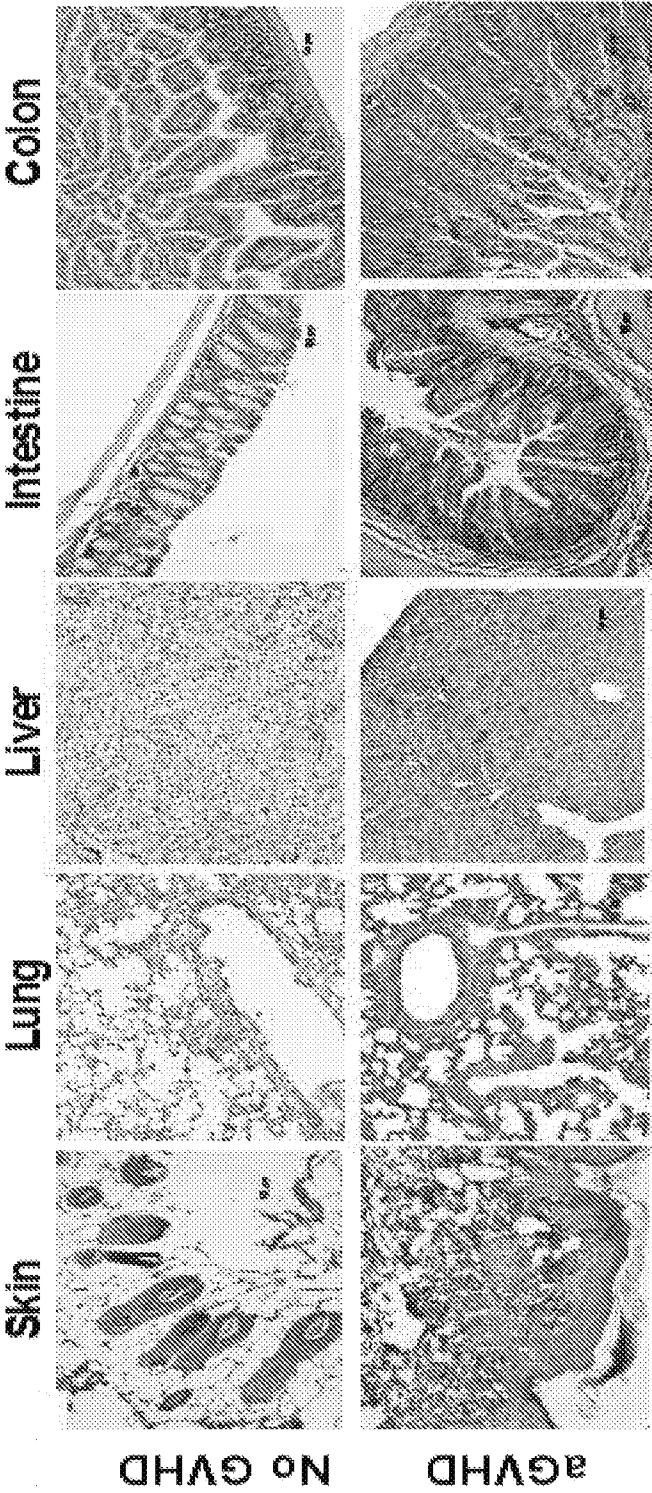


Figure 2

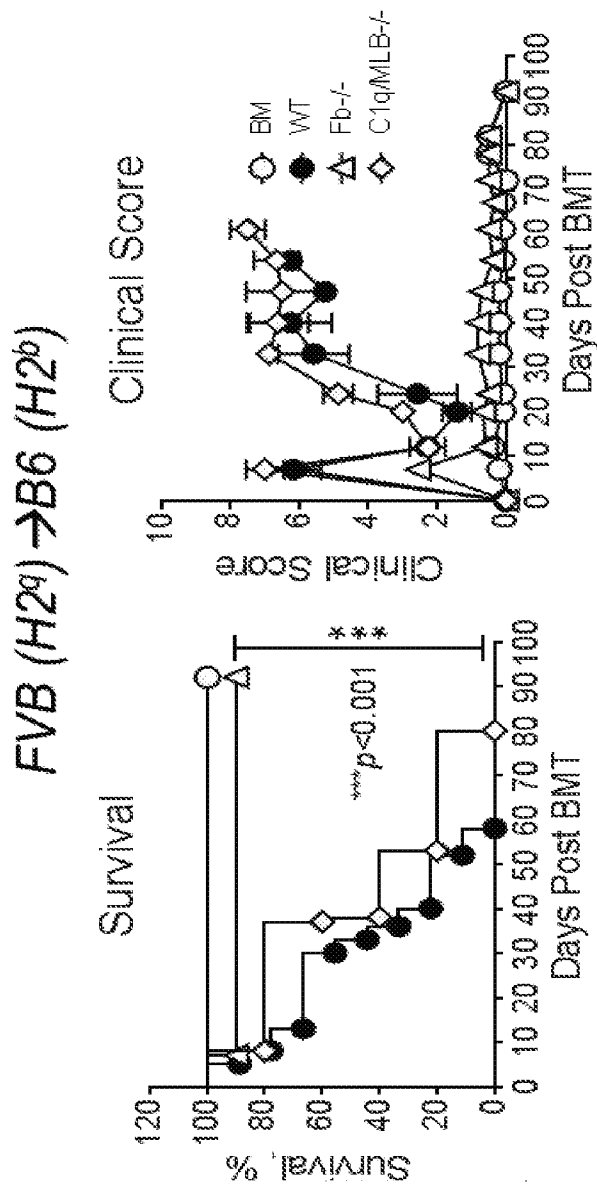


Figure 3

Fig. 4A

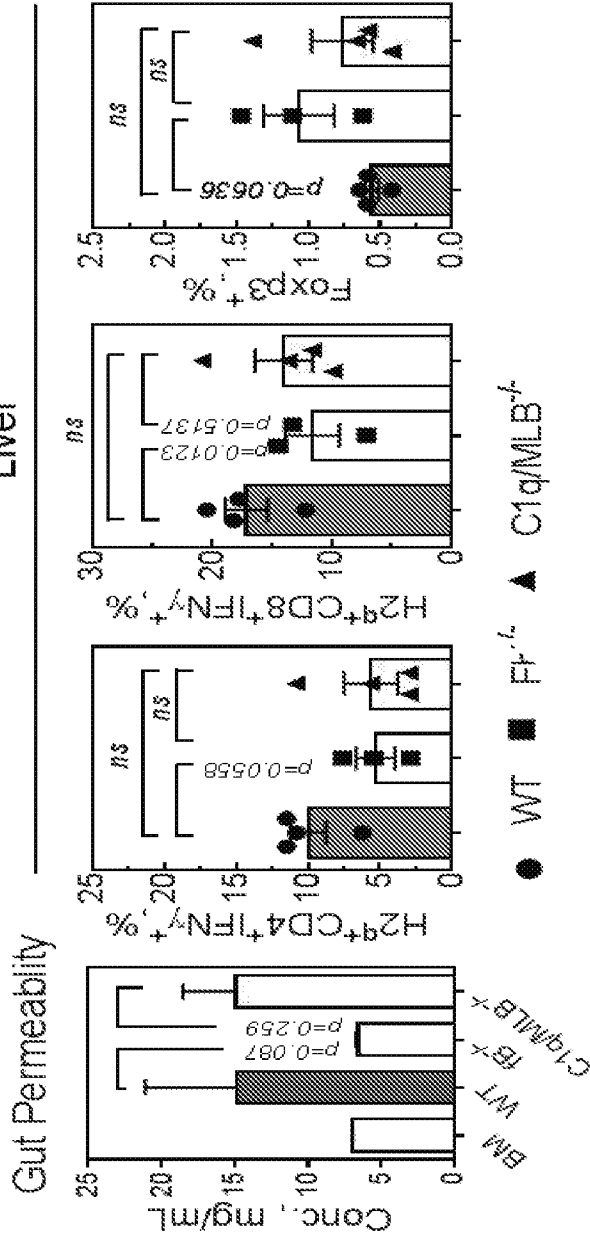


Fig. 4B

Fig. 4C

Spleen

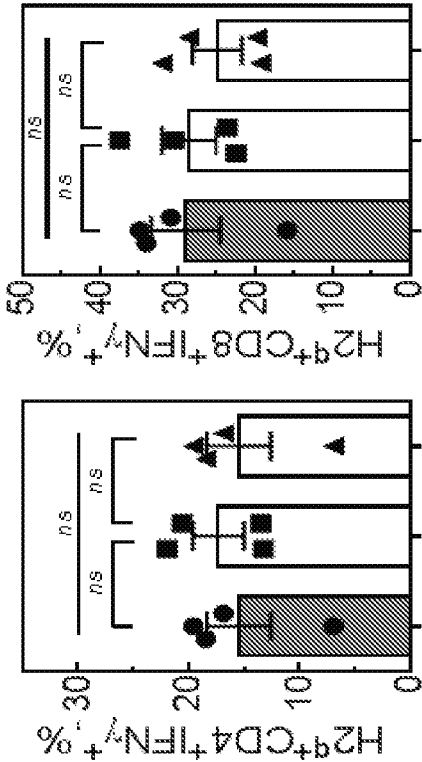


Figure 4

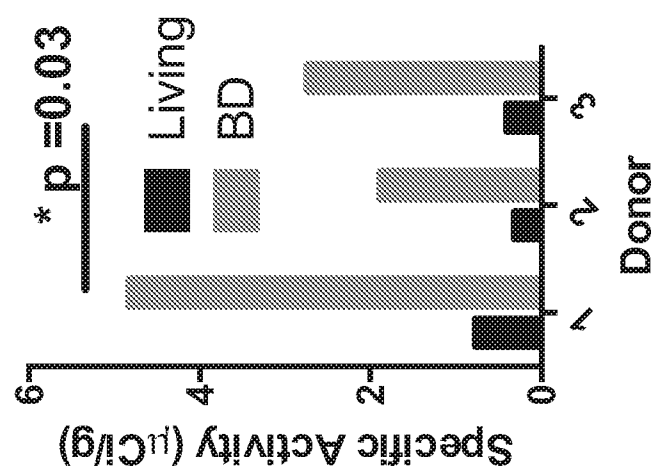


Figure 5

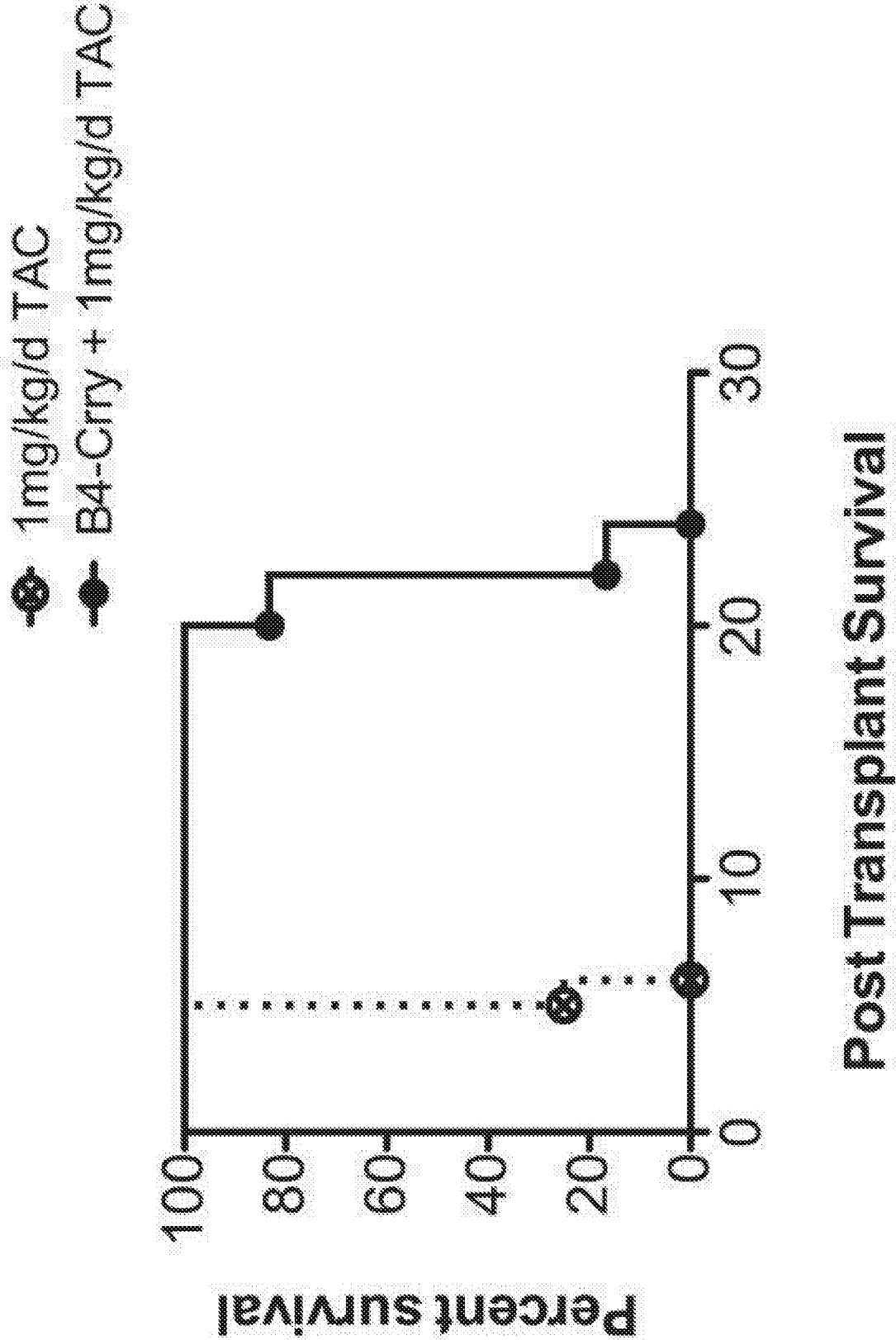


Figure 6

B4Crry nucleic acid sequence (SEQ ID NO: 61)

ATG TCC GTG CCT ACC CAG GTG CTC GGA CTC CTG CTG TGG CTC ACC GAC GCC AGG TGT GTG AAG CTG CAG GAG AGC GGA CCC GAG CTG GTG
 AAG CCT GGA GCC TCC GTG AAG ATC AGC TGC AAG GCT TCC GGA TAC ACC TTC ACC GAC TAC TAT ATG AAC TGG GTG AAG CAG AGC CAC GGC AAG
 AGC CTG GAG TGG ATC GGC GAC ATC AAC CCT AAC AAC GGC GGC ACC TCC TAC AAC CAG AAG TTC AAG GGC AAG GCT ACA CTG ACC GTG GAC AAG
 TCC TCC AGC ACC GCC TAC ATG GAG CTC AGG AGC CTG ACC TCC GAG GAT TCC GCC GTC TAT TAC TGT GCC CGG TAC GAC TAC GGC TGG TAT TTC GAC
 GTG TGG GGC CAG GGC ACA ACC GTC ACA GTC TCC AGC GGA GGA GGA AGC GGC GGC GGA GGA TCC GGA GGC GGA GGC GAT GTC CTG ATG
 ACA CAG ACA CCT CTG AGC CTC CCC GTG AGC CTG GGA GAC CAA GCC TCC ATC TCC TGC AGG TCC TCC CAG TCC ATC GTG CAC AGC AAT GGC AAC ACC
 TAC CTG GAG TGG TAT CTG CAG AAG CCT GGC CAG TCC CCC AAG CTG ATC TAC AAG GTG TCC AAC CGG TTC AGC GGC GTC CCT GAC AGG TTC
 TCC GGA TCC GGA AGC GGC ACA GAT TTC ACC CTG AAG ATC AGC AGG GTC GAG GCC GAG GAC CTG GGA GTG TAC TAC TGC TTC CAG GGC TCC CAT
 GTC CCT TAC ACC TTC GGC GGC ACC AAA CTG GAG ATC AAG CGG GGC GGA GGT GCG TCG GGT GGC GGC TCT TGC CCA GCC CCA TCA CAG
 CTT CCT TCT GCC AAA CCT ATA AAT CTA ACT GAT GAA TCC ATG TTT CCC ATT GGA ACA TAT TTG TTG TAT GAA TGT CTC CCA GGA TAT ATC AAG AGG
 CAG TTC TCT ATC ACC TGC AAA CAA GAC TCA ACC TGG ACG AGT GCT GAA GAT AAG TGT ATA CGA AAA CAA TGT AAA ACT CCT TCA GAT CCT GAG AAT
 GGC TTG GTA CAT GTA CAC ACA GGC ATT CAG TTT GGA TCC CGT ATT AAT TAT ACT TGT AAT CAA GGA TAC CGC CTC ATT GGT TCC TCC TCT GCT GTA
 TGT GTC ATC ACT GAT CAA AGT GTT GAT TGG GAT ACT GAG GCA CCT ATT TGT GAG TGG ATT CCT TGT GAG ATA CCC CCA GGC ATT CCC AAT GGA GAT
 TTC TTC AGT TCA ACC AGA GAA GAC TTT CAT TAT GGA ATG GTG GTT ACC TAC CGC TGC AAC ACT GAT GCG AGA GGG AAG GCG CTC TTT AAC CTG
 GTG GGT GAG CCC TCC TTA TAC TGT ACC AGC AAC GAT GGT GAA ATT GGA GTC TGG AGC GGC CCT CCT CAG TGC ATT GAA CTC AAC AAA TGT ACT
 CCT CCT CCC TAT GTT GAA AAT GCA GTC ATG CTG TCT GAG AAC AGA AGC TTG TTT TCC TTA AGG GAT ATT GTG GAG TTT AGA TGT CAC CCT GGC TTT
 ATC ATG AAA GGA GCC AGC AGT GTG CAT TGT CAG TCC CTA AAC AAA TGG GAG CCA GAG TTA CCA AGC TGC TTC AAG GGA GTG ATA TGT CGT CTC
 CCT CAG GAG ATG AGT GGA TTC CAG AAG GGG TTG GGA ATG AAA AAA GAA TAT TAT TAT GGA GAG AAT GTA ACC TTG GAA TGT GAG GAT GGG TAT
 ACT CTA GAA GGC AGT TCT CAA AGC CAG TGC CAG TCT GAT GGC AGC TGG AAT CCT CTT CTG GCC AAA TGT GTA TCT CGC TCA ATC ATC GAG GGC
 AGG CAT CAC CAC CAT CAC CAC TGA

B4Crry amino acid sequence (SEQ ID NO: 62)

MSVPTQVLGLLLWLTDARCVKLQESGPPELVKPGASVKISCKASGYTFTDYMNWVKQSHGKSLWIGDINPNNGGTSYNQKFKGKATLTVDKSSSTA
 YMELRSLTSEDSAVYVCARYDYAWYFDVWGQTTVTVSSGGGGSGGGGGGGLVMTQTPLSLPVSLGDAQISCRSSQSIHNSNGNTYLEWYLQ
 KPGQSPKLLIYKVSNRFGVDPDRFSGSGTDFTLKISRVEAEDLGVYFCFGSHVPYTFGGGTKEIKRGGGGGGGGGCPAPSQLPSAKPINLTDESM
 FPIGTYLLECLPGYIKRQFSITCKQDSTWTS AEDKCIKQCKTSPDPEGLVHVHTGIQFGSRINVTCTNQGRLIGSSSAVCVITDQSDVDWDEAPICEWI
 PCEIPPGIPNGDFFSSTREDFHYGMVVTYRCNTDARGKALFNLVGEPSLYCTSDNGEIGVWSGPPPPQCIELNKCCTPPVVENAVMLSENRSLSLRDIVE
 FRCHPGFIMKGASSVHCQSLNKEWPELPSCFKGVICRLPQEMSGFKGLGMKKEYYGENVTLECEDGYTLEGSSQCCQSDGSWNPLLAKCVSRSLI
 EGRHHHHHH

Figure 7

C2Crry amino acid sequence (SEQ ID NO: 63)

ATG AGC GTG CCT ACA CAG GTG CTC GGC CTG CTG AAG GCC CGG TGT GTG AAG CTG CAG TCC GGA ACC GAG CTG GTG AAA
 CCT GGC GCC AGC GTG AAA CTG AGC TGC AAA GCC AGC GGA TAC ACC TTC ACC TCC TAC TGG ATG CAC TGG GTG AAA CAG AGG CCT GGC CAG GGC CTG
 GAA TGG ATT GGC AAC ATC AAC CCC AGC AAC GGC GGC ACC AAC TAC AAT GAG AAG TTC AAG AGC AAG GCC ACC CTG ACC GTG GAT AAG TCC TCC TCC
 ACC GCC TAC ATG CAG CTG TCC TCC CTC ACC TCC CAG GAC AGC GCC GTC TAT TAC TGT GCC AGG CGG GGC ATC AGG CTG AGG CAC TTC GAC TAC TGG
 GGC CAA GGC ACA ACC GTC ACC GTG AGC TCC GGA GGA GGC AGC GGA GGC TCC GGC GGA GGC GGA AGC GAC ATT CAG ATG ACC CAG
 AGC CCC AAG TTC ATG TCC ACC TCC GTC GGC GAC AGG GTG AGC ATC ACC TGT AAG GCC AGC CAG GAT GTC GGC ACA GCT GTG GCC TGG TAC CAG CAG
 AAG CCC GGC CAG TCC CCC AAG CTG CTG ATC TAC TGG GCT TCC ACA AGG CAT ACC GGC GTC CCC GAT AGG TTC ACA GGC TCC GGC TCC GGC ACC GAC
 TTC ACA CTC ACC ATC AGC AAC GTC CAG TCC GAG GAC CTG GCC GAC TAC TTC TGC CAG CAG TAC TCC AGC TAC CCC CTC ACC TTC GGC GCT GGC ACC
 AAG CTG GAA CTC AAG CGG GGC GGA GGT GGT GGC GGC GGA TCT TGC CCA GCC CCA TCA CAG CTT CCT TCT GCC AAA CCT ATA AAT CTA ACT
 GAT GAA TCC ATG TTT CCC ATT GGA ACA TAT TTG TTG TAT GAA TGT CTC CCA GGA TAT ATC AAG AGG CAG TTC TCT ATC ACC TGC AAA CAA GAC TCA
 ACC TGG ACG AGT GCT GAA GAT AAG TGT ATA CGA AAA CAA TGT AAA ACT CCT TCA GAT CCT GAG AAT GGC TTG GTA CAT GTA CAC ACA GGC ATT CAG
 TTT GGA TCC CGT ATT AAT TAT ACT TGT AAT CAA GGA TAC CGC CTC ATT GGT TCC TCC TCT GCT GTA TGT GTC ATC ACT GAT CAA AGT GTT GAT TGG GAT
 ACT GAG GCA CCT ATT TGT GAG TGG ATT CCT TGT GAG ATA CCC CCA GGC ATT CCC AAT GGA GAT TTC TTC AGT TCA ACC AGA GAA GAC TTT CAT TAT
 GGA ATG GTG GTT ACC TAC CGC TGC AAC ACT GAT GCG AGA GGG AAG GCG CTC TTT AAC CTG GTG GGT GAG CCC TCC TTA TAC TGT ACC AGC AAC GAT
 GGT GAA ATT GGA GTC TGG AGC GGC CCT CCT CCT CAG TGC ATT GAA CTC AAC AAA TGT ACT CCT CCC TAT GTT GAA AAT GCA GTC ATG CTG TCT
 GAG AAC AGA AGC TTG TTT TCC TTA AGG GAT ATT GTG GAG TTT AGA TGT CAC CCT GGC TTT ATC ATG AAA GGA GCC AGC AGT GTG CAT TGT CAG TCC
 CTA AAC AAA TGG GAG CCA GAG TTA CCA AGC TGC TTC AAG GGA GTG ATA TGT CGT CTC CCT CAG GAG ATG AGT GGA TTC CAG AAG GGG TTG GGA ATG
 AAA AAA GAA TAT TAT TAT GGA GAG AAT GTA ACC TTG GAA TGT GAG GAT GGG TAT ACT CTA GAA GGC AGT TCT CAA AGC CAG TGC CAG TCT GAT GGC
 AGC TGG AAT CCT CTT CTG GCC AAA TGT GTA TCT CGC TCA ATC ATC GAG GGC AGG CAT CAC CAC CAT CAC CAC TGA TAG

C2Crry amino acid sequence (SEQ ID NO: 64)

MSVPTQVLGLLLWLTDARCVKLQESGTELVPKPGASVKLSCKASGYTFTSYWMHWVKQRPQGQGLEWIGNINPSNGGTNYNEKFKSKATLTVDKSSSTAYMQLS
 SLTSEDSAVVYVCARRGIRLRHFDYWGGTTVTSSGGGGGGGGGGSDIQMTQSPKFMSTSVGDRVSITCKASQDVGTAVAWYQQKPGQSPKLLIYWAS
 TRHTGVPDRFTGSGSGTDFTLTISNVQSEDLADYFCQQYSSYPLTFAGATKLELKRGGGGGGGSCAPSQLPSAKPINLTDESMFPIGTLYLLECLPGYIKRQFSI
 TCKQDSTWTSIEDKCIKQCKTPSDPENGLVHVHTGIQFGSRINVTNQQYRLIGSSSAVCVITDQSVWDWTEAPICEWIPCEIPPGIPNGDFFSSTREDFHYGM
 VVTYRCNTDARGKALFNLVGEPSLYCTSDNGEIGVWSGPPPPQCIELNCKTTPPPYVENAVMLSENRSLSLRDIVEFRCHPGFIMKGASSVHCQSLNKEPELPSC
 FKGVICRLPQEMSGFQKGLGMKKEYYYGENVTLECEDGYTLEGSSQSQSQSDGSWNPLLAKCVSRSIIEGRHHHHH

Figure 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/56929

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, Claims 1-4, directed to a composition.

Group II, claims 5-14, directed to a method of treating or preventing an injury associated with transplantation.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

*****Continued in the extra sheet*****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/56929

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 16/00; A61K 38/00; A61K 39/00; A61K 38/13; A61K 31/44 (2017.01)

CPC - C07K 2319/00; A61K 38/16; C07K 14/47; C07K 2317/622; C07K 16/18; A61K 38/13; C07K 7/645; C07D 491/04; A61K 31/436

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2016/0083469 A1 (THE REGENTS OF THE UNIVERSITY OF COLORADO, A BODY CORPORATE et al.) 24 March 2016 (24.03.2016), Abstract, para [0005], [0012], [0030], [0091], [0284], [0329], [0403], and [0426]	1-4
A	US 2013/0224109 A1 (STROM et al.) 29 August 2013 (29.08.2013), para [0107], and [0115]	1-4
A	US 2014/0335113 A1 (BAXTER HEALTHCARE SA et al.) 13 November 2014 (13.11.2014), Abstract, para [0005], [0007], [0008], [0039], [0040], [0042], [0064], [0070], [0087], [0091], and [0097]	1-4
A	SALVADORI et al., Update on ischemia-reperfusion injury in kidney transplantation: Pathogenesis and treatment. World J Transplant. 2015, Vol. 5(2), p. 52-67. Entire documentation, especially Abstract; pg 53, Introduction; pg 55, Fig 1; pg 57, col 2, para 2; pg 58, col 2, para 2; pg 60, Table 2; and pg 61, col 1, para 7, and col 2, para 5	1-4
A	ATKINSON et al., Targeting Pathogenic Postischemic Self-Recognition by Natural IgM to Protect Against Posttransplantation Cardiac Reperfusion Injury. Circulation. 2015, Vol. 131(13), p. 1171-80. Entire documentation, especially Abstract	1-4
A	ALAWIEH et al., Complement in the homeostatic and ischemic brain. Front Immunol. 2015, Vol. 6:417. PDF File: pg 1-18. Abstract; pg 1, Introduction; pg 2, col 1, middle para and Fig 1; pg 4, Fig 2; pg 10, col 1, para 1 and para 2; pg 12, col 1, last para, and col 2, last para; and pg 14, col 1, and col 2, up para	1-4
A	WO 2015/187992 A2 (THE REGENTS OF THE UNIVERSITY OF COLORADO, A BODY CORPORATE) 10 December 2015 (10.12.2015), Entire documentation, especially Abstract	1-4

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

18 December 2017

Date of mailing of the international search report

09 MAR 2018

Name and mailing address of the ISA/US

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P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/56929

Continuation of:
Box No III (unity of invention is lacking)

Special Technical Feature

Groups I-II are related as a product (Group I) and methods of potentially using the product (Group II).

Group I includes the special technical feature of a composition comprising (b) a sub-therapeutic dose of an immunosuppressant agent in combination with (a), not required by Group II.

Group II includes the special technical feature of administering to a subject in need thereof (claim 5) or to a transplant (claim 14) a therapeutically effective amount of a therapeutic agent, not required by Group I.

Common Technical Features

The inventions of Groups I-II share the technical feature of a composition for treating or preventing an injury associated with transplantation, comprising (a) a targeted inhibitor molecule wherein the targeted inhibitor molecule comprises a targeting portion and an inhibitor portion, wherein the molecule inhibits complement pathways (part of claim 1, and part of claim 5 or claim 14).

However, these shared technical features do not represent a contribution over prior art as being anticipated by US 2016/0083469 A1 to THE REGENTS OF THE UNIVERSITY OF COLORADO, A BODY CORPORATE et al. (hereinafter 'Univ_COLORADO') as follows:

Univ_COLORADO discloses a composition for treating or preventing an injury associated with transplantation (para [0030] - 'a composition comprising a construct, ... (i) an antibody ... and (ii) a therapeutic agent.... a complement inhibitor'; Abstract - 'provides targeted delivery... constructs for treating ... in vivo tissue injuries in an individual', wherein 'treating... injuries in an individual' comprising 'treating or preventing an injury associated with transplantation'; para [0403] - 'treatment... including ... tissue damage resulting from ischemia-reperfusion injury, ocular diseases ... hyperacute rejection in organ transplantation'; para [0005] - 'ischemia-reperfusion injury is important in ... post-transplantation injury and chronic rejection'; para [0426] - 'compositions ... used to treat and/or improve the outcome of ... corneal transplantation') comprising

(a) a targeted inhibitor molecule wherein the targeted inhibitor molecule comprises a targeting portion and an inhibitor portion (para [0030] - 'a composition comprising a construct, ... (i) an antibody ... binds to Annexin IV or phospholipid, and (ii) a therapeutic agent.... a complement inhibitor', wherein 'an antibody ... binds to Annexin IV' is 'a targeting portion'; Abstract - 'targeted delivery approach utilizes an antibody ... wherein the antibody or fragment thereof specifically binds to Annexin IV or phospholipid'; para [0012] - 'construct comprises (a) an antibody or a fragment thereof, wherein the antibody or a fragment thereof specifically binds to Annexin IV or a phospholipid; and (b) a complement inhibitor'; para [0426] - 'compositions ... used to treat and/or improve the outcome of ... corneal transplantation'),

--wherein the molecule inhibits complement pathways (para [0030] - 'a composition comprising a construct, ... (i) an antibody ... binds to Annexin IV or phospholipid, and (ii) a therapeutic agent.... a complement inhibitor', wherein 'a complement inhibitor' is 'the molecule inhibits complement pathways'; para [0091] - 'delivering a complement modulator (such as a complement inhibitor) ... to a site of tissue injury in an individual ... a composition comprising a construct, wherein the construct comprises (a) an antibody or a fragment thereof, ... specifically binds to Annexin IV ... and (b) a complement modulator (such as a complement inhibitor)').

Without a shared special technical feature, the inventions lack unity with one another.

Groups I-II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note:

I) Claim 3 contains multiple first appeared abbreviations each with no full name introduced.

II) Claim 4 contains the first appeared abbreviations of "FK506" and "mTOR", each with no full name introduced.