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(54) **LONG-ACTING COMPSTATIN ANALOGS AND RELATED COMPOSITIONS AND METHODS**

LANGWIRKENDE COMPSTATIN-ANALOGA SOWIE VERWANDTE ZUSAMMENSETZUNGEN UND
VERFAHREN

ANALOGUES DE LA COMPSTATINE À LONGUE DURÉE D'ACTION ET COMPOSITIONS ET
MÉTHODES ASSOCIÉES

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(73) Proprietor: **Apellis Pharmaceuticals, Inc.**
Waltham, MA 02451 (US)

(72) Inventors:
• **FRANCOIS, Cedric**
Prospect, KY 40059 (US)
• **DESCHATELETS, Pascal**
Prospect, KY 40059 (US)

(74) Representative: **J A Kemp LLP**
80 Turnmill Street
London EC1M 5QU (GB)

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Description

Background of the Invention

[0001] Complement is a system consisting of more than 30 plasma and cell-bound proteins that plays a significant role in both innate and adaptive immunity. The proteins of the complement system act in a series of enzymatic cascades through a variety of protein interactions and cleavage events. Complement activation occurs via three main pathways: the antibody-dependent classical pathway, the alternative pathway, and the mannose-binding lectin (MBL) pathway. Inappropriate or excessive complement activation is an underlying cause or contributing factor to a number of serious diseases and conditions, and considerable effort has been devoted over the past several decades to exploring various complement inhibitors as therapeutic agents. However, there remains a need for innovative approaches to inhibiting complement activation for a variety of therapeutic purposes.

Summary of the Invention

[0002] The invention provides compstatin analogs and compositions comprising such compstatin analogs, as defined in the claims. The compstatin analog of the invention is also referred to herein as a long-acting compstatin analog. The invention further provides a compstatin analog or composition comprising a compstatin analog as defined in the claims, for use as a medicament, for example in treating a complement-mediated disorder.

[0003] The compstatin analog or composition of the invention may be used in methods of protecting a cell or organ from complement-mediated damage. Such use may comprise contacting the cell with a cell-reactive compstatin analog. The cell can be any type of cell in For example, the cell may be a blood cell. The blood cell may be a red blood cell (RBC), also referred to as an erythrocyte. The cell may have abnormally low expression, surface density, and/or activity of one or more complement regulatory proteins. For example, the cell may have a mutation in a gene encoding such protein, wherein the mutation results in reduced or absent expression and/or reduced activity of the encoded protein. The cell can be of any animal type or species. For example, the cell can be mammalian, e.g., primate (human or a non-human primate), rodent (e.g., mouse, rat, rabbit), ungulate (e.g., pig, sheep, cow), canine, or feline. The protection may be from primate complement, e.g., human complement. The cell may be contacted ex vivo (outside the body of a subject). The cell may be contacted in vivo (in a subject, e.g., a human). The cell may be for transplantation into a subject or have been transplanted into a subject. Disclosed herein is an isolated cell having a compstatin analog covalently attached thereto. Also disclosed is an isolated tissue or organ having a compstatin analog attached to at least some of its cells.

[0004] The invention provides a compstatin analog or composition comprising a compstatin analog as defined in the claims for use in treating a subject in need of treatment for a complement-mediated disorder. Such use comprises administering the compstatin analog to the subject. In some embodiments, the complement-mediated disorder is paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), or another disorder associated with complement-mediated hemolysis. In some embodiments the disorder is ischemia/reperfusion (I/R) injury (e.g., due to myocardial infarction, thromboembolic stroke, or surgery). In some embodiments, the disorder is trauma. In some embodiments, the disorder is transplant rejection. In some embodiments the disorder is a chronic respiratory disorder, e.g., asthma or COPD.

[0005] The practice of certain aspects described herein may employ conventional techniques of molecular biology, cell culture, recombinant nucleic acid (e.g., DNA) technology, immunology, and/or nucleic acid and polypeptide synthesis, detection, manipulation, and quantification, etc., that are within the ordinary skill of the art. See, e.g., Ausubel, F., et al., (eds.), Current Protocols in Molecular Biology, Current Protocols in Immunology, Current Protocols in Protein Science, and Current Protocols in Cell Biology, all John Wiley & Sons, N.Y., e.g., edition current as of January 2010 or later; Sambrook, Russell, and Sambrook, Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 2001 or 4th ed, 2012.

Brief Description of the Drawing

[0006]

Figure 1 is a plot that shows percent complement activation inhibiting activity of compstatin analog CA28 (SEQ ID NO: 28) and three long-acting compstatin analogs (CA28-1, CA28-2, CA28-3), as a function of peptide concentration (μ M). Inhibition of complement activation was tested in vitro using a classical complement inhibition assay. The plot shows values obtained by averaging the results of two sets of measurements. CA28 (circles; red), CA28-1 (crosses (x); blue); CA28-2 (triangles, green), CA28-3 (squares (purple)).

Figure 2 is a plot that shows percent complement activation inhibiting activity of CA28 and long-acting compstatin analogs CA28-2 and CA28-3, as a function of compound concentration (μ M). CA28 (squares, light gray), CA28-2

(diamonds, black), CA28-3 (circles, dark gray). CA28-3 is a compound that contains multiple peptide moieties. Although the activity per peptide moiety is less than the activity of an individual CA28 molecule, the total activity of CA28-3 exceeds the activity of CA28 on a molar basis.

Figure 3 is a plot that shows plasma concentrations versus time of CA28 and long-acting compstatin analogs CA28-2 and CA28-3 in Cynomolgus monkeys following a single intravenous injection. CA28 was administered at 200 mg/kg. CA28-2 and CA28-3 were each administered at 50 mg/kg. In calculating the doses for these experiments the administered CA28-2 and CA28-3 substance was assumed to consist 80% of active compound w/w based on dry weight. However, during the sample analysis, the standard curve assumed a 100% of active compound w/w based on dry weight, by an estimated 30%. Thus, the values for C_{max} overestimate the actual C_{max}. CA28 (squares, light gray), CA28-2 (triangles, black), CA28-3 (circles, dark gray).

Figure 4 is a plot that shows percent complement activation inhibiting activity of CA28 and long-acting compstatin analogs CA28-4, as a function of compound concentration (μ M). Inhibition of complement activation was tested in vitro using a classical complement inhibition assay. The plot shows values obtained by averaging the results of four sets of measurements for CA28-4. CA28 (squares, light gray), CA28-4 (crosses, black).

Figure 5 is a plot that shows concentrations versus time of CA28 and long-acting compstatin analogs CA28-2, CA28-3, and CA28-4 in Cynomolgus monkeys following a single intravenous injection. CA28 was administered at 200 mg/kg. CA28-2, CA28-3, and CA28-4 were each administered at 50 mg/kg. In calculating the doses for these experiments the administered CA28-2 and CA28-3 substance was assumed to consist 80% of active compound w/w based on dry weight. However, during the sample analysis, the standard curve assumed a 100% of active compound w/w based on dry weight. Thus, the values for C_{max} overestimate the C_{max} that would be achieved if these compounds had been administered at the indicated doses on a dry mass basis, by an estimated 30%. CA28 (squares, light gray), CA28-2 (triangles, black), CA28-3 (circles, dark gray), CA28-4 (inverted triangles, black).

Figure 6 is a representative chromatogram showing ultraviolet (UV) detection of a PEG-based long-acting compstatin analog using reverse phase HPLC. The peak with a retention time (RT) of 33.68 minutes represents the PEGylated compstatin analog and had a relative area of 96%.

Figure 7 is a plot that shows percent complement activation inhibiting activity of CA28 and long-acting compstatin analogs, as a function of compound concentration (μ M). CA28-2CS (diamonds, red); CA28-2GS (crosses, blue); CA28-2HS (triangles, green); CA28-2TS (squares, black).

Figure 8 is a plot that shows percent complement activation inhibiting activity of CA28 and bifunctionalized long-acting compstatin analog, CA28-2GS-BF, as a function of compound concentration (micromoles). CA28 (open circles, blue); CA28-2G-SBF (filled circles, red).

Figure 9 is a plot that shows the plasma concentration versus time of CA28 and long-acting compstatin analog CA28-2GS-BF in Cynomolgus monkeys following either a single intravenous injection (CA28 (squares, red) and CA28-2GS-BF (circles, purple) or when administered by subcutaneous injection once daily for 7 days (CA28-2GS-BF only, asterisks, blue). CA28-2GSBF was administered at 25 mg/ml. Dosing volume was 2 ml/kg for IV and 0.28 ml/kg/day for the subcutaneous administration. Data for CA28 was from a different experiment in which the compound was also in 5% dextrose and was formulated as 20 mg/ml with a 10 ml/kg dosing volume. The vehicle in each case was 5% dextrose in water.

Figures 10(A) and 10(B) presents plots that show percent complement activation inhibiting activity of CA28 and bifunctionalized long-acting compstatin analog, CA28-2TS-BF, as a function of compound concentration (micromoles). (A) Classical pathway inhibition by CA28 (circles, red) and CA28-2TS-BF (crosses, blue). (B) Alternative pathway inhibition. CA28 (circles, red) and CA28-2TS-BF (crosses, blue).

Figure 10(C) (assuming a PEG moiety of 40 kD) shows the structure of CA28-2TS-BF.

Figure 11 is a plot that shows the plasma concentration versus time of CA28 and long-acting compstatin analog CA28-2TS-BF in Cynomolgus monkeys following a single intravenous injection of CA28 at 200 mg/kg (squares, red), a single intravenous injection of CA28-2TS-BF at 7 mg/kg (asterisks, purple), subcutaneous injection of CA28-2TS-BF at 7 mg/kg once only (circles, blue) or subcutaneous injection of CA28-2TS-BF at 7 mg/kg once daily for 7 consecutive days (inverted triangles, green). The vehicle in each case was 5% dextrose in water.

Figure 12 shows flow cytometric analysis of C3 deposition on red blood cells from a patient with PNH, which were exposed to activated complement in a modified Ham's test. (A) Results of a dilution experiment demonstrating the effect of CA28 on C3 deposition are shown. (B) Results of a dilution experiment demonstrating the effect of CA28-2GS-BF on C3 deposition are shown. Compound concentrations used are shown on and above each panel.

Figure 13 shows flow cytometric analysis of C3 deposition on red blood cells from a patient with PNH, which were exposed to activated complement in a modified Ham's test in the absence of complement inhibitors (left panel), in the presence of anti-C5 monoclonal antibody eculizumab (middle panel) and in the presence of CA28-2GS-BF (right panel).

Detailed Description of Certain Embodiments of the Invention

I. Definitions

[0007] The terms "approximately" or "about" in reference to a number generally include numbers that fall within $\pm 10\%$, in some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, in some embodiments $\pm 0.5\%$ of the number unless otherwise stated or otherwise evident from the context (except where such number would impermissibly exceed 100% of a possible value).

[0008] A "complement component" or "complement protein" is a protein that is involved in activation of the complement system or participates in one or more complement-mediated activities. Components of the classical complement pathway include, e.g., C1q, C1r, C1s, C2, C3, C4, C5, C6, C7, C8, C9, and the C5b-9 complex, also referred to as the membrane attack complex (MAC) and active fragments or enzymatic cleavage products of any of the foregoing (e.g., C3a, C3b, C4a, C4b, C5a, etc.). Components of the alternative pathway include, e.g., factors B, D, and properdin. Components of the lectin pathway include, e.g., MBL2, MASP-1, and MASP-2. Complement components also include cell-bound receptors for soluble complement components, wherein such receptor mediates one or more biological activities of such soluble complement component following binding of the soluble complement component. Such receptors include, e.g., C5a receptor (C5aR), C3a receptor (C3aR), Complement Receptor 1 (CR1), Complement Receptor 2 (CR2), Complement Receptor 3 (CR3, also known as CD45), etc. It will be appreciated that the term "complement component" is not intended to include those molecules and molecular structures that serve as "triggers" for complement activation, e.g., antigen-antibody complexes, foreign structures found on microbial or artificial surfaces, etc.

[0009] A "complement-mediated disorder" is any disorder in which complement activation is known or suspected of being a contributing and/or at least partially causative factor in at least some subjects suffering from the disorder, e.g., disorders in which complement activation results in tissue damage. Non-limiting examples of complement-mediated disorders include, but are not limited to, (i) various disorders characterized by hemolysis or hemolytic anemia such as atypical hemolytic uremic syndrome, cold agglutinin disease, paroxysmal nocturnal hemoglobinuria, transfusion reactions; (ii) transplant rejection (e.g., hyperacute or acute transplant rejection) or transplant dysfunction; (iii) disorders involving ischemia/reperfusion injury such as trauma, surgery (e.g., aneurysm repair), myocardial infarction, ischemic stroke; (iv) disorders of the respiratory system such as asthma and chronic obstructive pulmonary disease (COPD); (v) arthritis, e.g., rheumatoid arthritis; (vi) ocular disorders such as age-related macular degeneration (AMD), diabetic retinopathy, glaucoma, and uveitis. "Disorder" is used interchangeably herein with "disease", "condition", and similar words to refer to any impairment of health or state of abnormal functioning of an organism, e.g., any state in which medical and/or surgical management is indicated or for which a subject appropriately seeks medical and/or surgical attention. It should also be understood that the listing of a particular disorder within a particular category is for convenience and is not intended to limit the invention. It will be understood that certain disorders could appropriately be listed in multiple categories.

[0010] A "complement regulatory protein" is a protein involved in regulating complement activity. A complement regulatory protein may down-regulate complement activity by, e.g., inhibiting complement activation or by inactivating or accelerating decay of one or more activated complement proteins. Examples of complement regulatory proteins include C1 inhibitor, C4 binding protein, clusterin, vitronectin, CFH, factor I, and the cell-bound proteins CD46, CD55, CD59, CR1, CR2, and CR3.

[0011] "Isolated", as used herein, means 1) separated from at least some of the components with which it is usually associated in nature; 2) prepared or purified by a process that involves the hand of man; and/or 3) not occurring in nature, e.g., present in an artificial environment. In general, unless otherwise indicated or clearly evident, any entity, product, agent, composition, etc., may be deemed "isolated", if desired.

[0012] "Linked", as used herein with respect to two or more moieties, means that the moieties are physically associated or connected with one another to form a molecular structure that is sufficiently stable so that the moieties remain associated under the conditions in which the linkage is formed and, preferably, under the conditions in which the new molecular structure is used, e.g., physiological conditions. A linkage is preferably a covalent linkage. A linkage may be noncovalent. Moieties may be linked either directly or indirectly. When two moieties are directly linked, they are either covalently bonded to one another or are in sufficiently close proximity such that intermolecular forces between the two moieties maintain their association. When two moieties are indirectly linked, they are each linked either covalently or noncovalently to a third moiety, which maintains the association between the two moieties. In general, when two moieties are referred to as being linked by a "linking moiety" or "linking portion", the linkage between the two linked moieties is indirect, and typically each of the linked moieties is covalently bonded to the linking moiety. Two moieties may be linked using a "linker". A linker can be any suitable moiety that reacts with the entities to be linked within a reasonable period of time, under conditions consistent with stability of the entities (portions of which may be protected as appropriate, depending upon the conditions), and in sufficient amount, to produce a reasonable yield. Typically the linker will contain at least two functional groups, one of which reacts with a first entity and the other of which reacts with a second entity.

It will be appreciated that after the linker has reacted with the entities to be linked, the term "linker" may refer to the part of the resulting structure that originated from the linker, or at least the portion that does not include the reacted functional groups. A linking moiety may comprise a portion that does not participate in a bond with the entities being linked, and whose main purpose may be to spatially separate the entities from each other. Such portion may be referred to as a "spacer".

[0013] As used herein, "physiological conditions" refers to a set of conditions such as temperature, salt concentration, pH that at least in part mimic those conditions as typically found in a living subject, e.g., a mammalian subject. In some aspects, physiological conditions refer to conditions in an aqueous medium, e.g., a medium comprising at least 90%, 95%, 96%, 97%, 97%, 99%, or about 100% water on a volume/volume basis. Other liquids, if present, typically do not substantially affect protein secondary or tertiary structure. Usually, physiological conditions at least in part mimic those found in a body fluid such as blood or extracellular fluid, e.g., interstitial fluid, e.g., of a mammalian subject. A variety of physiological conditions useful for, e.g., in vitro assays, are known in the art. Generally, a medium under physiological conditions contains a physiological concentration of salt, e.g., sodium chloride. A physiological concentration of salt typically refers to a concentration ranging from about 250 mOsm/L to about 350 mOsm/L, e.g., about 275 mOsm/L to about 325 mOsm/L, e.g., about 300 mOsm/L. Typically, physiological conditions are approximately isotonic to a body fluid, e.g., blood or extracellular fluid, e.g., interstitial fluid. Typically, physiological conditions include a pH ranging from about 6.5 to about 7.8, e.g., about 7.0 to about 7.5. Typically, a physiological medium comprises a buffer substance that helps maintain the pH of the medium within a physiological range. Typically, physiological conditions comprise conditions such that a typical mammalian protein, e.g., a protein typically found in a body fluid, such as blood or extracellular fluid, substantially retains the secondary and, if applicable, tertiary structure that such protein has in the body fluid in which it is normally found. Components of a physiological medium are typically substantially nontoxic to mammalian cells at the concentration at which they are present in the physiological medium. A variety of physiological media (sometimes termed "buffers") are listed in various standard references, such as those cited above (e.g., Sambrook, et al., Protocols series). Typically, a physiological temperature ranges from about 25 degrees C to about 38 degrees C, e.g., from about 30 degrees C to about 37 degrees C, e.g., 35 degrees C to 37 degrees C.

[0014] "Polypeptide", as used herein, refers to a polymer of amino acids, optionally including one or more amino acid analogs. A protein is a molecule composed of one or more polypeptides. A peptide is a relatively short polypeptide, typically between about 2 and 60 amino acids in length, e.g., between 8 and 40 amino acids in length. The terms "protein", "polypeptide", and "peptide" may be used interchangeably. Polypeptides used herein may contain amino acids such as those that are naturally found in proteins, amino acids that are not naturally found in proteins, and/or amino acid analogs that are not amino acids. As used herein, an "analog" of an amino acid may be a different amino acid that structurally resembles the amino acid or a compound other than an amino acid that structurally resembles the amino acid. A large number of art-recognized analogs of the 20 amino acids commonly found in proteins (the "standard" amino acids) are known. One or more of the amino acids in a polypeptide may be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modification, etc. Certain non-limiting suitable analogs and modifications are described in WO2004026328 and/or below. The polypeptide may be acetylated, e.g., at the N-terminus and/or amidated, e.g., at the C-terminus.

[0015] The term "purified", as used herein, refers to substances that have been separated from at least some or most of the components with which they are associated in nature or when originally generated or with which they were associated prior to purification. In general, such purification involves action of the hand of man. Purified agents may be partially purified, substantially purified, or pure. Such agents may be, for example, at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more than 99% pure. A nucleic acid, polypeptide, or small molecule may be purified such that it constitutes at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more, of the total nucleic acid, polypeptide, or small molecule material, respectively, present in a preparation. An organic substance, e.g., a nucleic acid, polypeptide, or small molecule, may be purified such that it constitutes at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more, of the total organic material present in a preparation. Purity may be based on, e.g., dry weight, size of peaks on a chromatography tracing (GC, HPLC, etc.), molecular abundance, electrophoretic methods, intensity of bands on a gel, spectroscopic data (e.g., NMR), elemental analysis, high throughput sequencing, mass spectrometry, or any art-accepted quantification method. Water, buffer substances, ions, and/or small molecules (e.g., synthetic precursors such as nucleotides or amino acids), can optionally be present in a purified preparation. A purified agent may be prepared by separating it from other substances (e.g., other cellular materials), or by producing it in such a manner to achieve a desired degree of purity. Typically, "partially purified" with respect to a molecule produced by a cell means that a molecule produced by a cell is no longer present within the cell, e.g., the cell has been lysed and, optionally, at least some of the cellular material (e.g., cell wall, cell membrane(s), cell organelle(s)) has been removed and/or the molecule has been separated or segregated from at least some molecules of the same type (protein, RNA, DNA, etc.) that were present in the lysate.

[0016] "Recombinant host cells", "host cells", and other such terms, denote prokaryotic or eukaryotic cells or cell lines

that contain an exogenous nucleic acid (typically DNA) such as an expression vector comprising a nucleic acid that encodes a polypeptide of interest. It will be understood that such terms include the descendants of the original cell(s) into which the vector or other nucleic acid has been introduced. Appropriate host cells include any of those routinely used in the art for expressing polynucleotides (e.g., for purposes of producing polypeptide(s) encoded by such polynucleotides) including, for example, prokaryotes, such as *E. coli* or other bacteria such as species of *Escherichia*; *Lactobacillus*, *Bacillus* (e.g., *B. subtilis*), *Salmonella*, *Pseudomonas*, *Streptomyces*, *Staphylococcus*, etc; and eukaryotes, including for example, fungi, such as yeast (e.g., *Pichia* (e.g., *Pichia pastoris*), *Kluyveromyces*, such as *K. lactis*, *Hansenula*, e.g. *H. polymorpha*). Examples of other fungal cells are cells of filamentous fungi, e.g. *Aspergillus* spp., *Neurospora* spp., *Fusarium* spp. or *Trichoderma* spp., e.g., strains of *A. oryzae*, *A. nidulans* or *A. niger*; insect cells (e.g., Sf9), plant cells, and animal cells, e.g., mammalian cells such as CHO, R1.1, B-W, L-M, African Green Monkey Kidney cells (e.g. COS-1, COS-7, BSC-1, BSC-40 and BMT-10), and cultured human cells. Also encompassed are genetically modified cells in genetically modified (e.g., transgenic) plants or animals, wherein a recombinant polypeptide is produced by at least some such cells. A polypeptide may be secreted in milk, harvested from plant material, etc. The exogenous nucleic acid may be stably maintained as an episome such as a plasmid or may at least in part be integrated into the host cell's genome, optionally after being copied or reverse transcribed. Terms such as "host cells", etc., are also used to refer to cells or cell lines that can be used as recipients for an exogenous nucleic acid, prior to introduction of the nucleic acid. A "recombinant polynucleotide" generally is a polynucleotide that contains nucleic acid sequences that are not found joined directly to one another in nature. For example, the nucleic acid sequences may occur in different genes or different species or one or more of the sequence(s) may be a variant of a naturally occurring sequence or may at least in part be an artificial sequence that is not homologous to a naturally occurring sequence. A "recombinant polypeptide" generally is a polypeptide that is at least in part produced by transcription and translation of an exogenous nucleic acid by a recombinant host cell or by a cell-free in vitro expression system and/or that contains amino acid sequences that are not found joined directly to one another in nature. In the latter case, the recombinant polypeptide may be referred to as a "chimeric polypeptide". The amino acid sequences in a chimeric polypeptide may, for example, occur in different genes or in different species or one or more of the sequence(s) may be a variant of a naturally occurring sequence or may at least in part be an artificial sequence that is not identical or is not homologous to a naturally occurring sequence over a substantial portion of the length. It will be understood that a chimeric polypeptide may comprise two or more polypeptides. For example, first and second polypeptides A and B of a chimeric polypeptide may be directly linked (A-B or B-A) or may be separated by a third polypeptide portion C (A-C-B or B-C-A). Typically, portion C represents a polypeptide linker which may, for example, comprise multiple glycine and/or serine residues or any of a variety of other amino acids. Two or more polypeptides may be linked by non-polypeptide linker(s). "Recombinant" as used herein encompasses polypeptides produced by joining (e.g., chemically conjugating, enzymatically conjugating), shorter recombinant polypeptides that may be produced in recombinant host cells. A recombinant polypeptide may comprise a signal sequence that directs secretion of the polypeptide or a sequence that directs the expressed polypeptide to a specific compartment or organelle. Suitable sequences are known in the art. Appropriate sequences for a host cell type of interest (e.g., bacterial, fungal, mammalian, plant, etc.) may be selected. A signal sequence may be located at or near (e.g., within up to 10 - 50 amino acids of) the N-terminus or C-terminus. Typically, a polypeptide comprises a tag. A tag may be useful to facilitate detection and/or purification of a protein that contains it. Examples of tags include polyhistidine-tag (e.g., 6X-His tag), glutathione-S-transferase, maltose binding protein, NUS tag, SNUT tag, Strep tag, epitope tags such as V5, HA, Myc, or FLAG. A protease cleavage site may be located in the region between the tag and the polypeptide, allowing the polypeptide to be separated from the tag by exposure to the protease. A polynucleotide that encodes a recombinant polypeptide may be at least in part codon optimized for expression in a host cell of interest (e.g., bacterial, fungal, mammalian, plant, etc.). A tag may be located at or near (e.g., within up to 10 - 50 amino acids of) the N- or C-terminus of a polypeptide. A recombinant polypeptide may be isolated, purified, etc., using any of a variety of methods. See, e.g., Sambrook, Protocols series, or other standard references. Methods of use may include, e.g., dialysis (e.g., using membranes having defined pore size), chromatography, precipitation, gel purification, or affinity-based methods that may utilize a tag or a specific binding reagent such as an antibody.

[0017] "Reactive functional groups" as used herein refers to groups including, but not limited to, olefins, acetylenes, alcohols, phenols, ethers, oxides, halides, aldehydes, ketones, carboxylic acids, esters, amides, cyanates, isocyanates, thiocyanates, isothiocyanates, amines, hydrazines, hydrazones, hydrazides, diazo, diazonium, nitro, nitriles, mercaptans, sulfides, disulfides, sulfoxides, sulfones, sulfonic acids, sulfinic acids, acetals, ketals, anhydrides, sulfates, sulfenic acids, isonitriles, amidines, imides, imidates, nitrones, hydroxylamines, oximes, hydroxamic acids, thiohydroxamic acids, alkenes, ortho esters, sulfites, enamines, ynamines, ureas, pseudoureas, semicarbazides, carbodiimides, carbamates, imines, azides, azo compounds, azoxy compounds, and nitroso compounds, N-hydroxysuccinimide esters, maleimides, sulfhydryls, and the like. Methods to prepare each of these functional groups are well known in the art and their application to or modification for a particular purpose is within the ability of one of skill in the art (see, for example, Sandler and Karo, eds. ORGANIC FUNCTIONAL GROUP PREPARATIONS, Academic Press, San Diego, 1989, and Hermanson, G., Bioconjugate Techniques, 2nd ed., Academic Press, San Diego, 2008).

[0018] "Specific binding" generally refers to a physical association between a target polypeptide (or, more generally, a target molecule) and a binding molecule such as an antibody or ligand. The association is typically dependent upon the presence of a particular structural feature of the target such as an antigenic determinant, epitope, binding pocket or cleft, recognized by the binding molecule. For example, if an antibody is specific for epitope A, the presence of a polypeptide containing epitope A or the presence of free unlabeled A in a reaction containing both free labeled A and the binding molecule that binds thereto, will reduce the amount of labeled A that binds to the binding molecule. It is to be understood that specificity need not be absolute but generally refers to the context in which the binding occurs. For example, it is well known in the art that numerous antibodies cross-react with other epitopes in addition to those present in the target molecule. Such cross-reactivity may be acceptable depending upon the application for which the antibody is to be used. One of ordinary skill in the art will be able to select antibodies or ligands having a sufficient degree of specificity to perform appropriately in any given application (e.g., for detection of a target molecule, for therapeutic purposes, etc). It is also to be understood that specificity may be evaluated in the context of additional factors such as the affinity of the binding molecule for the target versus the affinity of the binding molecule for other targets, e.g., competitors. If a binding molecule exhibits a high affinity for a target molecule that it is desired to detect and low affinity for nontarget molecules, the antibody will likely be an acceptable reagent. Once the specificity of a binding molecule is established in one or more contexts, it may be employed in other, preferably similar, contexts without necessarily re-evaluating its specificity. Typically, the affinity (as measured by the equilibrium dissociation constant, Kd) of two molecules that exhibit specific binding is 10^{-3} M or less, e.g., 10^{-4} M or less, e.g., 10^{-5} M or less, e.g., 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, or 10^{-9} M or less under the conditions tested, e.g., under physiological conditions.

[0019] A "subject" treated according to the instant invention is typically a human, a non-human primate, or a lower animal (e.g., a mouse or rat), which expresses or contains at least some primate (e.g., human) complement component C3 and, optionally, one or more additional primate complement component(s). In some embodiments the subject is male. In some embodiments the subject is female. In some embodiments the subject is an adult, e.g., a human at least 18 years of age, e.g., between 18 and 100 years of age. In some embodiments, a human subject is at least 12 years of age. In some embodiments a subject is an adult, e.g., a human at least 18 years of age, e.g., between 18 and 100 years of age. In some embodiments a subject is at least 40, 45, 50, 55, 60, 65, 70, 75, or 80 years of age. In some embodiments the subject is a child, e.g., a human between 0 and 4 years of age, or between 5 and 11 years of age.

[0020] "Treating", as used herein in regard to treating a subject, refers to providing treatment, i.e., providing any type of medical or surgical management of a subject. The treatment can be provided in order to reverse, alleviate, inhibit the progression of, prevent or reduce the likelihood of a disease, or in order to reverse, alleviate, inhibit or prevent the progression of, prevent or reduce the likelihood of one or more symptoms or manifestations of a disease. "Prevent" refers to causing a disease or symptom or manifestation of a disease not to occur for at least a period of time in at least some individuals. Treating can include administering a compound or composition to the subject following the development of one or more symptoms or manifestations indicative of a disease, e.g., in order to reverse, alleviate, reduce the severity of, and/or inhibit or prevent the progression of the disease and/or to reverse, alleviate, reduce the severity of, and/or inhibit or one or more symptoms or manifestations of the disease. A compound or composition can be administered to a subject who has developed a disease, or is at increased risk of developing the disease relative to a member of the general population. A compound or composition can be administered to a subject who has developed a disease and is at increased risk of developing one or more particular symptoms or manifestations of the disease or an exacerbation of the disease relative to other individuals diagnosed with the disease, or relative to the subject's typical or average risk for such symptom or manifestation or exacerbation. For example, the subject may have been exposed to a "trigger" that places the subject at increased risk (e.g., temporarily increased risk) of experiencing an exacerbation. A compound or composition can be administered prophylactically, i.e., before development of any symptom or manifestation of the disease. Typically in this case the subject will be at risk of developing the disease, e.g., relative to a member of the general population, optionally matched in terms of age, sex, and/or other demographic variable(s).

[0021] A "vector" may be any of a variety of nucleic acid molecules, viruses, or portions thereof that are capable of mediating entry of, e.g., transferring, transporting, etc., a nucleic acid of interest between different genetic environments or into a cell. The nucleic acid of interest may be linked to, e.g., inserted into, the vector using, e.g., restriction and ligation. Vectors include, for example, DNA or RNA plasmids, cosmids, naturally occurring or modified viral genomes or portions thereof, nucleic acids that can be packaged into viral capsids, mini-chromosomes, artificial chromosomes, etc. Plasmid vectors typically include an origin of replication (e.g., for replication in prokaryotic cells). A plasmid may include part or all of a viral genome (e.g., a viral promoter, enhancer, processing or packaging signals, and/or sequences sufficient to give rise to a nucleic acid that can be integrated into the host cell genome and/or to give rise to infectious virus). Viruses or portions thereof that can be used to introduce nucleic acids into cells may be referred to as viral vectors. Viral vectors include, e.g., adenoviruses, adeno-associated viruses, retroviruses (e.g., lentiviruses, vaccinia virus and other poxviruses, herpesviruses (e.g., herpes simplex virus), and others. Baculovirus are of use, e.g., in insect cells. A wide range of plant viral vectors are known and include, e.g., those based on or comprising Cauliflower Mosaic Virus, Tobacco Mosaic Virus, or one or more genetic elements thereof (e.g., Cauliflower Mosaic Virus 35S promoter). Viral

vectors may or may not contain sufficient viral genetic information for production of infectious virus when introduced into host cells, i.e., viral vectors may be replication-competent or replication-defective. For example, where sufficient information for production of infectious virus is lacking, it may be supplied by a host cell or by another vector introduced into the cell, e.g., if production of virus is desired. Alternatively, such information may not be supplied, e.g., if production of virus is not desired. A nucleic acid to be transferred may be incorporated into a naturally occurring or modified viral genome or a portion thereof or may be present within a viral capsid as a separate nucleic acid molecule. A vector may contain one or more nucleic acids encoding a marker suitable for identifying and/or selecting cells that have taken up the vector. Markers include, for example, various proteins that increase or decrease either resistance or sensitivity to antibiotics or other agents (e.g., a protein that confers resistance to an antibiotic such as puromycin, hygromycin or blasticidin), enzymes whose activities are detectable by assays known in the art (e.g., β -galactosidase or alkaline phosphatase), and proteins or RNAs that detectably affect the phenotype of cells that express them (e.g., fluorescent proteins). Vectors often include one or more appropriately positioned sites for restriction enzymes, which may be used to facilitate insertion into the vector of a nucleic acid, e.g., a nucleic acid to be expressed. An expression vector is a vector into which a desired nucleic acid has been inserted or may be inserted such that it is operably linked to regulatory elements (also termed "regulatory sequences", "expression control elements", or "expression control sequences") and may be expressed as an RNA transcript (e.g., an mRNA that can be translated into protein or a noncoding RNA). Expression vectors include regulatory sequence(s), e.g., expression control sequences, sufficient to direct transcription of an operably linked nucleic acid under at least some conditions; other elements required or helpful for expression may be supplied by, e.g., the host cell or by an in vitro expression system. Such regulatory sequences typically include a promoter and may include enhancer sequences or upstream activator sequences. A vector may include sequences that encode a 5' untranslated region and/or a 3' untranslated region, which may comprise a cleavage and/or polyadenylation signal. In general, regulatory elements may be contained in a vector prior to insertion of a nucleic acid whose expression is desired or may be contained in an inserted nucleic acid or may be inserted into a vector following insertion of a nucleic acid whose expression is desired. As used herein, a nucleic acid and regulatory element(s) are said to be "operably linked" when they are covalently linked so as to place the expression or transcription of the nucleic acid under the influence or control of the regulatory element(s). For example, a promoter region would be operably linked to a nucleic acid if the promoter region were capable of effecting transcription of that nucleic acid. One of ordinary skill in the art will be aware that the precise nature of the regulatory sequences useful for gene expression may vary between species or cell types, but may in general include, as appropriate, sequences involved with the initiation of transcription, RNA processing, or initiation of translation. The choice and design of an appropriate vector and regulatory element(s) is within the ability and discretion of one of ordinary skill in the art. For example, one of skill in the art will select an appropriate promoter (or other expression control sequences) for expression in a desired species (e.g., a prokaryotic (bacterial) or eukaryotic (e.g., fungal, plant, mammalian species) or cell type. A vector may contain a promoter capable of directing expression in mammalian cells, such as a suitable viral promoter, e.g., from a cytomegalovirus (CMV), retrovirus, simian virus (e.g., SV40), papilloma virus, herpes virus or other virus that infects mammalian cells, or a mammalian promoter from, e.g., a gene such as EF1 α , ubiquitin (e.g., ubiquitin B or C), globin, actin, phosphoglycerate kinase (PGK), etc., or a composite promoter such as a CAG promoter (combination of the CMV early enhancer element and chicken beta-actin promoter). A human promoter may be used. A promoter that ordinarily directs transcription by a eukaryotic RNA polymerase I (a "pol I promoter"), e.g., (a U6, H1, 7SK or tRNA promoter or a functional variant thereof) may be used. A promoter that ordinarily directs transcription by a eukaryotic RNA polymerase II (a "pol II promoter") or a functional variant thereof may be used. A promoter that ordinarily directs transcription by a eukaryotic RNA polymerase III (a "pol III promoter"), e.g., a promoter for transcription of ribosomal RNA (other than 5S rRNA) or a functional variant thereof may be used. One of ordinary skill in the art will select an appropriate promoter for directing transcription of a sequence of interest. Examples of expression vectors that may be used in mammalian cells include, e.g., the pcDNA vector series, pSV2 vector series, pCMV vector series, pRSV vector series, pEF1 vector series, Gateway[®] vectors, etc. Typically, regulatable (e.g., inducible or repressible) expression control element(s), e.g., a regulatable promoter, is/are used so that expression can be regulated, e.g., turned on or increased or turned off or decreased. A vector may comprise a polynucleotide sequence that encodes a polypeptide, wherein the polynucleotide sequence is positioned in frame with a nucleic acid inserted into the vector so that an N- or C-terminal fusion is created. A polypeptide encoded by the polynucleotide sequence may comprise a signal sequence (which directs secretion of a protein) or a sequence that directs the expressed protein to a specific organelle or location in the cell such as the nucleus or mitochondria. A polypeptide may comprise a tag. A tag may be useful to facilitate detection and/or purification of a protein that contains it. Examples of tags include polyhistidine-tag (e.g., 6X-His tag), glutathione-S-transferase, maltose binding protein, NUS tag, SNUT tag, Strep tag, epitope tags such as V5, HA, Myc, or FLAG. A protease cleavage site may be located in the region between the protein encoded by the inserted nucleic acid and the polypeptide, allowing the polypeptide to be removed by exposure to the protease. Vectors may be introduced into host cells using methods known in the art. One of ordinary skill will select an appropriate method based, e.g., on the vector, cell type, etc. Examples of suitable methods include, e.g., calcium phosphate-mediated transfection, transfection with any of a variety of commercially available reagents, e.g., lipid-based

or non-lipid based, such as FuGENE, Lipofectamine, TurboFect; electroporation; microparticle bombardment, etc. Such methods are explained in detail in standard references such as Sambrook, Protocols series, and others.

[0022] As used herein the term "aliphatic" denotes a hydrocarbon moiety that may be straight-chain (i.e., unbranched), branched, or cyclic (including fused, bridging, and spiro-fused polycyclic) and may be completely saturated or may contain one or more units of unsaturation, but which is not aromatic. Unless otherwise specified, aliphatic groups contain 1-30 carbon atoms. In some embodiments, aliphatic groups contain 1-10 carbon atoms. In other embodiments, aliphatic groups contain 1-8 carbon atoms. In still other embodiments, aliphatic groups contain 1-6 carbon atoms, and in yet other embodiments aliphatic groups contain 1-4 carbon atoms. Suitable aliphatic groups include, but are not limited to, linear or branched, alkyl, alkenyl, and alkynyl groups, and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

[0023] As used herein, "alkyl" refers to a saturated straight, branched, or cyclic hydrocarbon having from about 1 to about 22 carbon atoms (and all combinations and subcombinations of ranges and specific numbers of carbon atoms therein), with from about 1 to about 12, or about 1 to about 7 carbon atoms being preferred in certain embodiments of the invention. Alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, n-pentyl, cyclopentyl, isopentyl, neopentyl, n-hexyl, isohexyl, cyclohexyl, cyclooctyl, adamantyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl.

[0024] As used herein, "halo" refers to F, Cl, Br or I.

[0025] As used herein, "alkanoyl" refers to an optionally substituted straight or branched aliphatic acyclic residue having about 1 to 10 carbon atoms (and all combinations and subcombinations of ranges and specific number of carbon atoms) therein, e.g., from about 1 to 7 carbon atoms which, as will be appreciated, is attached to a terminal C=O group with a single bond (and may also be referred to as an "acyl group"). Alkanoyl groups include, but are not limited to, formyl, acetyl, propionyl, butyryl, isobutyryl, pentanoyl, isopentanoyl, 2-methyl-butyryl, 2,2-dimethoxypropionyl, hexanoyl, heptanoyl, octanoyl, and the like, and for purposes of the present invention a formyl group is considered an alkanoyl group. "Lower alkanoyl" refers to an optionally substituted straight or branched aliphatic acyclic residue having about 1 to about 5 carbon atoms (and all combinations and subcombinations of ranges and specific number of carbon atoms). Such groups include, but are not limited to, formyl, acetyl, propionyl, butyryl, isobutyryl, pentanoyl, isopentanoyl, etc.

[0026] As used herein, "aryl" refers to an optionally substituted, mono- or bicyclic aromatic ring system having from about 5 to about 14 carbon atoms (and all combinations and subcombinations of ranges and specific numbers of carbon atoms therein), with from about 6 to about 10 carbons being preferred. Non-limiting examples include, for example, phenyl and naphthyl.

[0027] As used herein, "aralkyl" refers to alkyl radicals bearing an aryl substituent and having from about 6 to about 22 carbon atoms (and all combinations and subcombinations of ranges and specific numbers of carbon atoms therein), with from about 6 to about 12 carbon atoms being preferred in certain embodiments. Aralkyl groups can be optionally substituted. Non-limiting examples include, for example, benzyl, naphthylmethyl, diphenylmethyl, triphenylmethyl, phenylethyl, and diphenylethyl.

[0028] As used herein, the terms "alkoxy" and "alkoxyl" refer to an optionally substituted alkyl-O- group wherein alkyl is as previously defined. Exemplary alkoxy and alkoxyl groups include methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, and heptoxy.

[0029] As used herein, "carboxy" refers to a -C(=O)OH group.

[0030] As used herein, "alkoxycarbonyl" refers to a -C(=O)O-alkyl group, where alkyl is as previously defined.

[0031] As used herein, "aroyl" refers to a -C(=O)-aryl group, wherein aryl is as previously defined. Exemplary aroyl groups include benzoyl and naphthoyl.

[0032] The term "cyclic ring system" refers to an aromatic or non-aromatic, partially unsaturated or fully saturated, 3- to 10-membered ring system, which includes single rings of 3 to 8 atoms in size and bi- and tri-cyclic ring systems which may include aromatic 5- or 6-membered aryl or aromatic heterocyclic groups fused to a non-aromatic ring. These heterocyclic rings include those having from 1 to 3 heteroatoms independently selected from the group consisting of oxygen, sulfur, and nitrogen. In certain embodiments, the term heterocyclic refers to a non-aromatic 5-, 6-, or 7-membered ring or a polycyclic group wherein at least one ring atom is a heteroatom selected from the group consisting of O, S, and N, including, but not limited to, a bi- or tri-cyclic group, comprising fused six-membered rings having between one and three heteroatoms independently selected from the group consisting of the oxygen, sulfur, and nitrogen. In some embodiments, "cyclic ring system" refers to a cycloalkyl group which, as used herein, refers to groups having 3 to 10, e.g., 4 to 7 carbon atoms. Cycloalkyls include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like, which, is optionally substituted. In some embodiments, "cyclic ring system" refers to a cycloalkenyl or cycloalkynyl moiety, which is optionally substituted.

[0033] Typically, substituted chemical moieties include one or more substituents that replace hydrogen. Exemplary substituents include, for example, halo, alkyl, cycloalkyl, aralkyl, aryl, sulfhydryl, hydroxyl (-OH), alkoxyl, cyano (-CN), carboxyl (-COOH), -C(=O)O-alkyl, aminocarbonyl (-C(=O)NH₂), -N-substituted aminocarbonyl (-C(=O)NHR), CF₃, CF₂CF₃, and the like. In relation to the aforementioned substituents, each moiety R can be, independently, any of H,

alkyl, cycloalkyl, aryl, or aralkyl, for example.

[0034] As used herein, "L-amino acid" refers to any of the naturally occurring levorotatory alpha-amino acids normally present in proteins or the alkyl esters of those alpha-amino acids. The term "D-amino acid" refers to dextrorotatory alpha-amino acids. Unless specified otherwise, all amino acids referred to herein are L-amino acids.

[0035] As used herein, an "aromatic amino acid" is an amino acid that comprises at least one aromatic ring, e.g., it comprises an aryl group.

[0036] As used herein, an "aromatic amino acid analog" is an amino acid analog that comprises at least one aromatic ring, e.g., it comprises an aryl group.

II. Overview

[0037] As described in more detail herein, the invention provides a compstatin analog comprising a linear PEG moiety having a molecular weight of 40 kD and coupled to each of two compstatin analog moieties; wherein: (i) each compstatin analog moiety is a CA28-AEEAc-Lys moiety; (ii) the linear PEG moiety is coupled to the Lys side chain of each CA28-AEEAc-Lys moiety via an amide bond; and (iii) each CA28-AEEAc-Lys moiety is Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-[NH-CH₂CH₂OCH₂CH₂OCH₂-C(=O)]-Lys-NH₂ (SEQ ID NO: 51); wherein the Cys* groups are joined by a disulfide bond. The invention also provides a compstatin analog obtainable by coupling two CA28-AEEAc-Lys moieties to a bifunctional linear PEG moiety having a molecular weight of 40 kDa; wherein: (i) the bifunctional linear PEG moiety is coupled to the Lys side chain of each CA28-AEEAc-Lys moiety via an amide bond; and (ii) each CA28-AEEAc-Lys moiety is Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-[NH-CH₂CH₂OCH₂CH₂OCH₂-C(=O)]-Lys-NH₂ (SEQ ID NO: 51); wherein the Cys* groups are joined by a disulfide bond. Typically in such compstatin analogs the bifunctional linear PEG moiety is of Formula IVa as defined in the claims. Compositions comprising such compstatin analogs and methods of medical use of such compstatin analogs are also provided.

[0038] Cell-reactive compstatin analogs are compounds that comprise a compstatin analog moiety and a cell-reactive functional group that is capable of reacting with a functional group exposed at the surface of a cell, e.g., under physiological conditions, to form a covalent bond. The cell-reactive compstatin analog thus becomes covalently attached to the cell. Without wishing to be bound by any particular theory, a cell-tethered compstatin analog may protect the cell from complement-mediated damage by, for example, binding to C3 (which may be in the form of C3(H₂O)) at the cell surface and/or in the vicinity of the cell and inhibiting C3 cleavage and activation, and/or by binding to C3b and inhibiting its deposition on the cell or participation in the complement activation cascade. Isolated cells may be contacted with a cell-reactive compstatin analog ex vivo (outside the body). Such cells may be present in an isolated tissue or organ, e.g., a tissue or organ to be transplanted into a subject. Cells may be contacted with a cell-reactive compstatin analog in vivo, by administering the cell-reactive compstatin analog to a subject. The cell-reactive compstatin analog becomes covalently attached to cells in vivo. Accordingly, cells, tissues, and/or organs may be protected from the deleterious effects of complement activation for at least two weeks, without need for retreatment during that time.

[0039] Compstatin analogs comprising a targeting moiety that binds non-covalently to a target molecule present at the surface of cells or tissues or to an extracellular substance not attached to cells or tissues may be referred to as "targeted compstatin analogs". Often the target molecule is a protein or carbohydrate attached to the cell membrane and exposed at the cell surface. The targeting moiety targets the compstatin analog to a cell, tissue, or location susceptible to complement activation. Isolated cells may be contacted with a targeted compstatin analog ex vivo (outside the body). The cells may be present in an isolated tissue or organ, e.g., a tissue or organ to be transplanted into a subject. A targeted compstatin analog may be administered to a subject and become non-covalently attached to a cell, tissue, or extracellular substance in vivo. Accordingly, cells, tissues, and/or organs may be protected from the deleterious effects of complement activation for at least two weeks, without need for retreatment during that time. A targeted compstatin analog may comprise both a targeting moiety and a cell-reactive moiety. The targeting moiety targets the compstatin analog, e.g., to a particular cell type, by binding non-covalently to a molecule on such cells. The cell-reactive moiety then binds covalently to the cell or extracellular substance. Alternatively, a targeted compstatin analog may not comprise a cell-reactive moiety.

III. Complement System

[0040] In order to facilitate understanding of the invention, and without intending to limit the invention in any way, this section provides an overview of complement and its pathways of activation. Further details are found, e.g., in Kuby Immunology, 6th ed., 2006; Paul, W.E., Fundamental Immunology, Lippincott Williams & Wilkins; 6th ed., 2008; and Walport M.J., Complement. First of two parts. N Engl JMed., 344(14): 1058-66, 2001.

[0041] Complement is an arm of the innate immune system that plays an important role in defending the body against infectious agents. The complement system comprises more than 30 serum and cellular proteins that are involved in

three major pathways, known as the classical, alternative, and lectin pathways. The classical pathway is usually triggered by binding of a complex of antigen and IgM or IgG antibody to C1 (though certain other activators can also initiate the pathway). Activated C1 cleaves C4 and C2 to produce C4a and C4b, in addition to C2a and C2b. C4b and C2a combine to form C3 convertase, which cleaves C3 to form C3a and C3b. Binding of C3b to C3 convertase produces C5 convertase,

which cleaves C5 into C5a and C5b. C3a, C4a, and C5a are anaphylotoxins and mediate multiple reactions in the acute inflammatory response. C3a and C5a are also chemotactic factors that attract immune system cells such as neutrophils. **[0042]** The alternative pathway is initiated by and amplified at, e.g., microbial surfaces and various complex polysaccharides. In this pathway, hydrolysis of C3 to C3(H₂O), which occurs spontaneously at a low level, leads to binding of factor B, which is cleaved by factor D, generating a fluid phase C3 convertase that activates complement by cleaving C3 into C3a and C3b. C3b binds to targets such as cell surfaces and forms a complex with factor B, which is later cleaved by factor D, resulting in a C3 convertase. Surface-bound C3 convertases cleave and activate additional C3 molecules, resulting in rapid C3b deposition in close proximity to the site of activation and leading to formation of additional C3 convertase, which in turn generates additional C3b. This process results in a cycle of C3 cleavage and C3 convertase formation that significantly amplifies the response. Cleavage of C3 and binding of another molecule of C3b to the C3 convertase gives rise to a C5 convertase. C3 and C5 convertases of this pathway are regulated by host cell molecules CR1, DAF, MCP, CD59, and fH. The mode of action of these proteins involves either decay accelerating activity (i.e., ability to dissociate convertases), ability to serve as cofactors in the degradation of C3b or C4b by factor I, or both. Normally the presence of complement regulatory proteins on host cell surfaces prevents significant complement activation from occurring thereon.

[0043] The C5 convertases produced in both pathways cleave C5 to produce C5a and C5b. C5b then binds to C6, C7, and C8 to form C5b-8, which catalyzes polymerization of C9 to form the C5b-9 membrane attack complex (MAC). The MAC inserts itself into target cell membranes and causes cell lysis. Small amounts of MAC on the membrane of cells may have a variety of consequences other than cell death.

[0044] The lectin complement pathway is initiated by binding of mannose-binding lectin (MBL) and MBL-associated serine protease (MASP) to carbohydrates. The MB1-1 gene (known as LMAN-1 in humans) encodes a type I integral membrane protein localized in the intermediate region between the endoplasmic reticulum and the Golgi. The MBL-2 gene encodes the soluble mannose-binding protein found in serum. In the human lectin pathway, MASP-1 and MASP-2 are involved in the proteolysis of C4 and C2, leading to a C3 convertase described above.

[0045] Complement activity is regulated by various mammalian proteins referred to as complement control proteins (CCPs) or regulators of complement activation (RCA) proteins (U.S. Pat. No. 6,897,290). These proteins differ with respect to ligand specificity and mechanism(s) of complement inhibition. They may accelerate the normal decay of convertases and/or function as cofactors for factor I, to enzymatically cleave C3b and/or C4b into smaller fragments. CCPs are characterized by the presence of multiple (typically 4-56) homologous motifs known as short consensus repeats (SCR), complement control protein (CCP) modules, or SUSHI domains, about 50-70 amino acids in length that contain a conserved motif including four disulfide-bonded cysteines (two disulfide bonds), proline, tryptophan, and many hydrophobic residues. The CCP family includes complement receptor type 1 (CR1; C3b:C4b receptor), complement receptor type 2 (CR2), membrane cofactor protein (MCP; CD46), decay-accelerating factor (DAF), complement factor H (fH), and C4b-binding protein (C4bp). CD59 is a membrane-bound complement regulatory protein unrelated structurally to the CCPs. Complement regulatory proteins normally serve to limit complement activation that might otherwise occur on cells and tissues of the mammalian, e.g., human host. Thus, "self" cells are normally protected from the deleterious effects that would otherwise ensue were complement activation to proceed on these cells. Deficiencies or defects in complement regulatory protein(s) are involved in the pathogenesis of a variety of complement-mediated disorders, e.g., as discussed herein.

IV. Compstatin Analogs

[0046] Compstatin is a cyclic peptide that binds to C3 and inhibits complement activation. U.S. Pat. No. 6,319,897 describes a peptide having the sequence Ile- [Cys-Val-Val-Gln-Asp-Trp-Gly-His-His-Arg-Cys]-Thr (SEQ ID NO: 1), with the disulfide bond between the two cysteines denoted by brackets. It will be understood that the name "compstatin" was not used in U.S. Pat. No. 6,319,897 but was subsequently adopted in the scientific and patent literature (see, e.g., Morikis, et al., Protein Sci., 7(3):619-27, 1998) to refer to a peptide having the same sequence as SEQ ID NO: 2 disclosed in U.S. Pat. No. 6,319,897, but amidated at the C terminus as shown in Table 1 (SEQ ID NO: 8). The term "compstatin" is used herein consistently with such usage (i.e., to refer to SEQ ID NO: 8). Compstatin analogs that have higher complement inhibiting activity than compstatin have been developed. See, e.g., WO2004/026328 (PCT/US2003/029653), Morikis, D., et al., Biochem Soc Trans. 32(Pt 1):28-32, 2004, Mallik, B., et al., J. Med. Chem., 274-286, 2005; Katragadda, M., et al. J. Med. Chem., 49: 4616-4622, 2006; WO2007062249 (PCT/US2006/045539); WO2007044668 (PCT/US2006/039397), WO/2009/046198 (PCT/US2008/078593); WO/2010/127336 (PCT/US2010/033345) and discussion below.

[0047] Compstatin analogs may be acetylated or amidated, e.g., at the N-terminus and/or C-terminus. For example, compstatin analogs may be acetylated at the N-terminus and amidated at the C-terminus. Consistent with usage in the art, "compstatin" as used herein, and the activities of compstatin analogs described herein relative to that of compstatin, refer to compstatin amidated at the C-terminus (Mallik, 2005, *supra*).

[0048] Concatamers or multimers of compstatin or a complement inhibiting analog thereof are also disclosed.

[0049] The invention provides compstatin analogs comprising specific compstatin analog moieties as defined in the claims. The term "compstatin analog" generally includes compstatin and any complement inhibiting analog thereof. The term "compstatin analog" typically encompasses compstatin and other compounds designed or identified based on compstatin and whose complement inhibiting activity is at least 50% as great as that of compstatin as measured, e.g., using any complement activation assay accepted in the art or substantially similar or equivalent assays. Certain suitable assays are described in U.S. Pat. No. 6,319,897, WO2004/026328, Morikis, *supra*, Mallik, *supra*, Katragadda 2006, *supra*, WO2007062249 (PCT/US2006/045539); WO2007044668 (PCT/US2006/039397), WO/2009/046198 (PCT/US2008/078593); and/or WO/2010/127336 (PCT/US2010/033345). The assay may, for example, measure alternative or classical pathway-mediated erythrocyte lysis or be an ELISA assay. An assay described in WO/2010/135717 (PCT/US2010/035871) may be used.

[0050] The activity of a compstatin analog may be expressed in terms of its IC_{50} (the concentration of the compound that inhibits complement activation by 50%), with a lower IC_{50} indicating a higher activity as recognized in the art. It is noted that certain modifications are known to reduce or eliminate complement inhibiting activity. The IC_{50} of compstatin has been measured as 12 μ M using an alternative pathway-mediated erythrocyte lysis assay (WO2004/026328). It will be appreciated that the precise IC_{50} value measured for a given compstatin analog will vary with experimental conditions (e.g., the serum concentration used in the assay). Comparative values, e.g., obtained from experiments in which IC_{50} is determined for multiple different compounds under substantially identical conditions, are of use. The IC_{50} of a compstatin analog may be no more than the IC_{50} of compstatin. The activity of a compstatin analog may be between 2 and 99 times that of compstatin (i.e., the analog may have an IC_{50} that is less than the IC_{50} of compstatin by a factor of between 2 and 99). For example, the activity may be between 10 and 50 times as great as that of compstatin, or between 50 and 99 times as great as that of compstatin. The activity of a compstatin analog may be between 99 and 264 times that of compstatin. For example, the activity may be 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, or 264 times as great as that of compstatin. The activity may be between 250 and 300, 300 and 350, 350 and 400, or 400 and 500 times as great as that of compstatin. Compstatin analogs may have activities between 500 and 1000 times that of compstatin, or more. The IC_{50} of a compstatin analog may be between about 0.2 μ M and about 0.5 μ M. The IC_{50} of a compstatin analog may be between about 0.1 μ M and about 0.2 μ M. The IC_{50} of a compstatin analog may be between about 0.05 μ M and about 0.1 μ M. The IC_{50} of a compstatin analog may be between about 0.001 μ M and about 0.05 μ M.

[0051] The K_d of compstatin binding to C3 can be measured using isothermal titration calorimetry (Katragadda, et al., J. Biol. Chem., 279(53), 54987-54995, 2004). Binding affinity of a variety of compstatin analogs for C3 has been correlated with their activity, with a lower K_d indicating a higher binding affinity, as recognized in the art. A linear correlation between binding affinity and activity was shown for certain analogs tested (Katragadda, 2004, *supra*; Katragadda 2006, *supra*). A compstatin analog may bind to C3 with a K_d of between 0.1 μ M and 1.0 μ M, between 0.05 μ M and 0.1 μ M, between 0.025 μ M and 0.05 μ M, between 0.015 μ M and 0.025 μ M, between 0.01 μ M and 0.015 μ M, or between 0.001 μ M and 0.01 μ M.

[0052] Compounds "designed or identified based on compstatin" may include compounds that comprise an amino acid chain whose sequence is obtained by (i) modifying the sequence of compstatin (e.g., replacing one or more amino acids of the sequence of compstatin with a different amino acid or amino acid analog, inserting one or more amino acids or amino acid analogs into the sequence of compstatin, or deleting one or more amino acids from the sequence of compstatin); (ii) selection from a phage display peptide library in which one or more amino acids of compstatin is randomized, and optionally further modified according to method (i); or (iii) identified by screening for compounds that compete with compstatin or any analog thereof obtained by methods (i) or (ii) for binding to C3 or a fragment thereof. Many useful compstatin analogs comprise a hydrophobic cluster, a β -turn, and a disulfide bridge.

[0053] Compstatin analogs may comprise or consist essentially of a sequence that is obtained by making 1, 2, 3, or 4 substitutions in the sequence of compstatin, i.e., 1, 2, 3, or 4 amino acids in the sequence of compstatin is replaced by a different standard amino acid or by a non-standard amino acid. The amino acid at position 4 may be altered. The amino acid at position 9 may be altered. The amino acids at positions 4 and 9 may be altered. Only the amino acids at positions 4 and 9 may be altered. The amino acid at position 4 or 9 may be altered, or both amino acids 4 and 9 may be altered, and in addition up to 2 amino acids located at positions selected from 1, 7, 10, 11, and 13 may be altered. The amino acids at positions 4, 7, and 9 may be altered. Amino acids at position 2, 12, or both may be altered, provided that the alteration preserves the ability of the compound to be cyclized. Such alteration(s) at positions 2 and/or 12 may be in addition to the alteration(s) at position 1, 4, 7, 9, 10, 11, and/or 13. Optionally the sequence of any compstatin analog whose sequence is obtained by replacing one or more amino acids of compstatin sequence may further include

up to 1, 2, or 3 additional amino acids at the C-terminus. The additional amino acid may be Gly. Optionally the sequence of any of compstatin analog whose sequence is obtained by replacing one or more amino acids of compstatin sequence further includes up to 5, or up to 10 additional amino acids at the C-terminus. A disclosed compstatin analog may comprise or consist essentially of a sequence identical to that of compstatin except at positions corresponding to positions 4 and 9 in the sequence of compstatin.

[0054] Compstatin and certain compstatin analogs having somewhat greater activity than compstatin typically contain only standard amino acids ("standard amino acids" are glycine, leucine, isoleucine, valine, alanine, phenylalanine, tyrosine, tryptophan, aspartic acid, asparagine, glutamic acid, glutamine, cysteine, methionine, arginine, lysine, proline, serine, threonine and histidine). Certain compstatin analogs having improved activity incorporate one or more non-standard amino acids. Non-standard amino acids include singly and multiply halogenated (e.g., fluorinated) amino acids, D-amino acids, homo-amino acids, N-alkyl amino acids, dehydroamino acids, aromatic amino acids (other than phenylalanine, tyrosine and tryptophan), ortho-, meta- or para-aminobenzoic acid, phospho-amino acids, methoxylated amino acids, and α,α -disubstituted amino acids. A compstatin analog may be designed by replacing one or more L-amino acids with the corresponding D-amino acid. Exemplary non-standard amino acids include 2-naphthylalanine (2-Nal), 1-naphthylalanine (1-Nal), 2-indanylglycine carboxylic acid (2Ig1), dihydrotryptophan (Dht), 4-benzoyl-L-phenylalanine (Bpa), 2- α -aminobutyric acid (2-Abu), 3- α -aminobutyric acid (3-Abu), 4- α -aminobutyric acid (4-Abu), cyclohexylalanine (Cha), homocyclohexylalanine (hCha), 4-fluoro-L-tryptophan (4fW), 5-fluoro-L-tryptophan (5fW), 6-fluoro-L-tryptophan (6fW), 4-hydroxy-L-tryptophan (4OH-W), 5-hydroxy-L-tryptophan (5OH-W), 6-hydroxy-L-tryptophan (6OH-W), 1-methyl-L-tryptophan (1MeW), 4-methyl-L-tryptophan (4MeW), 5-methyl-L-tryptophan (5MeW), 7-aza-L-tryptophan (7aW), α -methyl-L-tryptophan (α MeW), β -methyl-L-tryptophan (β MeW), N-methyl-L-tryptophan (NMeW), ornithine (orn), citrulline, nor-leucine, γ -glutamic acid, etc.

[0055] In the invention, the compstatin analog comprises a Trp analog at position 4 relative to the sequence of compstatin. Exemplary Trp analogs are mentioned above. See also Beene, et. al. Biochemistry 41: 10262-10269, 2002 (describing, *inter alia*, singly- and multiply-halogenated Trp analogs); Babitzke & Yanofsky, J. Biol. Chem. 270: 12452-12456, 1995 (describing, *inter alia*, methylated and halogenated Trp and other Trp and indole analogs); and U.S. Patents 6,214,790, 6,169,057, 5,776,970, 4,870,097, 4,576,750 and 4,299,838. Other Trp analogs include variants that are substituted (e.g., by a methyl group) at the α or β carbon and, optionally, also at one or more positions of the indole ring. Amino acids comprising two or more aromatic rings, including substituted, unsubstituted, or alternatively substituted variants thereof, are of interest as Trp analogs. In the invention the Trp analog at position 4 is 1-methyl-tryptophan. Other disclosed Trp analogs include those comprising a 1-alkyl substituent, e.g., a lower alkyl (e.g., C₁-C₅) substituent; N(α) methyl tryptophan; 5-methyltryptophan; and analogs comprising a 1-alkanoyl substituent, e.g., a lower alkanoyl (e.g., C₁-C₅), such as 1-acetyl-L-tryptophan and L- β -tryptophan.

[0056] In the invention, the Trp analog has increased hydrophobic character relative to Trp as the indole ring is substituted by methyl. The Trp analog may participate in a hydrophobic interaction with C3.

[0057] Other disclosed Trp analogs may comprise a substituted or unsubstituted bicyclic aromatic ring component or two or more substituted or unsubstituted monocyclic aromatic ring components. Still other disclosed Trp analogs have increased propensity to form hydrogen bonds with C3 relative to Trp but do not have increased hydrophobic character relative to Trp; for example a Trp analog may have increased polarity relative to Trp and/or an increased ability to participate in an electrostatic interaction with a hydrogen bond donor on C3. Such Trp analogs with an increased hydrogen bond forming character may comprise an electronegative substituent on the indole ring.

[0058] Some disclosed compstatin analogs comprise one or more Ala analogs (e.g., at position 9 relative to the sequence of compstatin), e.g., Ala analogs that are identical to Ala except that they include one or more CH₂ groups in the side chain; for example an unbranched single methyl amino acid such as 2-Abu. Some disclosed compstatin analogs comprise one or more Trp analogs (e.g., at position 4 and/or 7 relative to the sequence of compstatin) and an Ala analog (e.g., at position 9 relative to the sequence of compstatin).

[0059] The compstatin analog of the invention comprises a peptide which has a core sequence of Gln - Asp - Xaa - Gly, where Xaa is Trp. Absent evidence to the contrary, one of skill in the art would recognize that any non-naturally occurring peptide whose sequence comprises this core sequence and that inhibits complement activation and/or binds to C3 will have been designed based on the sequence of compstatin. The Gln - Asp - Xaa - Gly peptide can form a β -turn.

[0060] In more detail, the compstatin analog of the invention comprises a peptide which has a core sequence of X'aa-Gln - Asp - Xaa - Gly (SEQ ID NO: 3), where X'aa is 1-methyltryptophan and Xaa is Trp. The core sequence forms a β -turn in the context of the peptide. The β -turn may be flexible, allowing the peptide to assume two or more conformations as assessed for example, using nuclear magnetic resonance (NMR). X'aa has increased hydrophobic character relative to Trp.

[0061] In further detail, the compstatin analog of the invention comprises a peptide which has a core sequence of X'aa-Gln - Asp - Xaa - Gly-X'aa (SEQ ID NO: 4), where X'aa is 1-methyltryptophan and Xaa is Trp; and X'aa is Ala.

[0062] In the invention the peptide is cyclic. The peptide is cyclized via a bond between two amino acids, one of which is (X'aa)_n and the other of which is located within (X'aa)_m as defined herein. In the invention the cyclic portion of the

peptide is 11 amino acids in length, with a disulfide bond between amino acids at positions 2 and 12. A peptide which is 13 amino acids long, with a bond between amino acids at positions 2 and 12 results in a cyclic portion 11 amino acids in length.

[0063] In still further detail the compstatin analog of the invention comprises a peptide comprising the sequence X'aa1 - X'aa2 - X'aa3 - X'aa4 -Gln-Asp-Xaa-Gly- X"aa1- X"aa2-X"aa3- X"aa4- X"aa5 (SEQ ID NO: 5). In the invention, as defined in the claims, X'aa4 is 1-methyltryptophan and Xaa is Trp, and X'aa1, X'aa2, X'aa3, X"aa1, X"aa2, X"aa3, X"aa4, and X"aa5 are amino acids as defined in the claims. In the invention, X"aa1 is Ala. The peptide is cyclized via a covalent bond between X'aa2 and X"aa4. The covalently bound amino acid are each Cys and the covalent bond is a disulfide (S-S) bond. Other disclosed covalent bonds include C-C, C-O, C-S, and C-N bonds.

[0064] Also disclosed herein are compounds in which one of the covalently bound residues is an amino acid or amino acid analog having a side chain that comprises a primary or secondary amine, the other covalently bound residue is an amino acid or amino acid analog having a side chain that comprises a carboxylic acid group, and the covalent bond is an amide bond. Amino acids or amino acid analogs having a side chain that comprises a primary or secondary amine include lysine and diaminocarboxylic acids of general structure $\text{NH}_2(\text{CH}_2)_n\text{CH}(\text{NH}_2)\text{COOH}$ such as 2,3-diaminopropionic acid (dapa), 2,4-diaminobutyric acid (daba), and ornithine (orn), wherein $n = 1$ (dapa), 2 (daba), and 3 (orn), respectively. Examples of amino acids having a side chain that comprises a carboxylic acid group include dicarboxylic amino acids such as glutamic acid and aspartic acid, and analogs such as beta-hydroxy-L-glutamic acid. A peptide may be cyclized with a thioether bond, e.g., as described in PCT/US2011/052442 (WO/2012/040259). For example, a disulfide bond may be replaced with a thioether bond. A cystathionine may be formed. The cystathionine may be a delta-cystathionine or a gamma-cystathionine.

[0065] A disclosed modification comprises replacement of a Cys-Cys disulfide bond between cysteines at X'aa2 and X"aa4 in SEQ ID NO: 5 (or corresponding positions in other sequences) with addition of a CH_2 , to form a homocysteine at X'aa2 or X"aa4, and introduction of a thioether bond, to form a cystathionine, such as a gamma-cystathionine or a delta-cystathionine. Another disclosed modification comprises replacement of the disulfide bond with a thioether bond without the addition of a CH_2 , thereby forming a lantithionine. A compstatin analog having a thioether in place of a disulfide bond may have increased stability, at least under some conditions, as compared with the compstatin analog having the disulfide bond.

[0066] The compstatin analog of the invention comprises a peptide having a sequence:

[0067] Xaa1 - Cys - Val - Xaa2 - Gln - Asp - Xaa2* - Gly - Xaa3 - His - Arg - Cys - Xaa4 (SEQ ID NO: 6); wherein:

Xaa1 is B^1 -Ile and B^1 represents a first blocking moiety;

Xaa2 is 1-methyltryptophan and Xaa2* is Trp;

Xaa3 is Ala;

Xaa4 is L-Thr; and

the two Cys residues are joined by a disulfide bond.

[0068] The blocking moiety stabilizes the a peptide against degradation that would otherwise occur in mammalian (e.g., human or non-human primate) blood or interstitial fluid. In the invention, the blocking moiety B^1 is an acetyl group, i.e., Xaa1 is Ac-Ile. In other disclosed compstatin analogs a carboxy terminal -OH of any of the L-Thr, D-Thr, Ile, Val, Gly, Ala, or Asn at position Xaa4 optionally is replaced by a second blocking moiety B^2 .

[0069] In the invention, as defined further herein, the compstatin analog is both acetylated at the N-terminus and amidated at the C-terminus.

[0070] The compstatin analog of the invention thus comprises a peptide having a sequence:

[0071] Xaa1 - Cys - Val - Xaa2 - Gln - Asp - Xaa2* - Gly - Xaa3 - His - Arg - Cys - Xaa4 (SEQ ID NO: 7); wherein:

Xaa1 is Ac-Ile;

Xaa2 is 1-methyl tryptophan and Xaa2* is Trp;

Xaa3 is Ala;

Xaa4 is L-Thr; and the two Cys residues are joined by a disulfide bond.

[0072] Table 1 provides further disclosed compstatin analogs. The analogs are referred to in abbreviated form in the left column by indicating specific modifications at designated positions (1-13) as compared to the parent peptide, compstatin. Consistent with usage in the art, "compstatin" as used herein, and the activities of compstatin analogs described herein relative to that of compstatin, refer to the compstatin peptide amidated at the C-terminus. Unless otherwise indicated, peptides in Table 1 are amidated at the C-terminus. Bold text is used to indicate certain modifications. Activity relative to compstatin is based on published data and assays described therein (WO2004/026328, WO2007044668, Mallik, 2005; Katragadda, 2006). Where multiple publications reporting an activity were consulted, the more recently published value is used, and it will be recognized that values may be adjusted in the case of differences between assays.

It will also be appreciated that the peptides listed in Table 1 are cyclized via a disulfide bond between the two Cys residues when used in therapeutic compositions and methods. Alternate means for cyclizing the peptides are disclosed above. One or more amino acid(s) of any of the compstatin analogs disclosed in Table 1 can be an N-alkyl amino acid (e.g., an N-methyl amino acid). For example, and without limitation, at least one amino acid within the cyclic portion of the peptide, at least one amino acid N-terminal to the cyclic portion, and/or at least one amino acid C-terminal to the cyclic portion may be an N-alkyl amino acid, e.g., an N-methyl amino acid. For example, disclosed compstatin analogs may comprises an N-methyl glycine, e.g., at the position corresponding to position 8 of compstatin and/or at the position corresponding to position 13 of compstatin. One or more of the compstatin analogs in Table 1 may contain at least one N-methyl glycine, e.g., at the position corresponding to position 8 of compstatin and/or at the position corresponding to position 13 of compstatin. One or more of the compstatin analogs in Table 1 may contain at least one N-methyl isoleucine, e.g., at the position corresponding to position 13 of compstatin. For example, a Thr at or near the C-terminal end of a peptide whose sequence is listed in Table 1 or any other compstatin analog sequence may be replaced by N-methyl Ile. The N-methylated amino acids may comprise N-methyl Gly at position 8 and N-methyl Ile at position 13. The N-methylated amino acids may comprise N-methyl Gly in a core sequence such as SEQ ID NO: 3 or SEQ ID NO: 4. The N-methylated amino acids may comprise N-methyl Gly in a core sequence such as SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 7.

Table 1

Peptide	Sequence	SEQ ID NO:	Activity over compstatin
Compstatin	<i>H</i> -ICVVQDWGHHRCT-CONH ₂	8	*
Ac-compstatin	Ac-ICVVQDWGHHRCT-CONH ₂	9	3xmore
Ac-V4Y/H9A	Ac-ICVYQDWGAHRCT-CONH ₂	10	14xmore
Ac-V4W/H9A -OH	Ac-ICVWQDWGAHRCT-COOH	11	27xmore
Ac-V4W/H9A	Ac-ICVWQDWGAHRCT-CONH ₂	12	45xmore
Ac-V4W/H9A/T13dT -OH	Ac-ICVWQDWGAHRCdT-COOH	13	55xmore
Ac-V4(2-Nal)/H9A	Ac-ICV(2-Nal)QDWGAHRCT-CONH ₂	14	99xmore
Ac V4(2-Nal)/H9A -OH	Ac-ICV(2-Nal)QDWGAHRCT-COOH	15	38xmore
Ac V4(1-Nal)/H9A -OH	Ac-ICV(1-Nal)QDWGAHRCT-COOH	16	30xmore
Ac-V42Igl/H9A	Ac-ICV(2-Igl)QDWGAHRCT-CONH ₂	17	39xmore
Ac-V42Igl/H9A -OH	Ac-ICV(2-Igl)QDWGAHRCT-COOH	18	37xmore
Ac-V4Dht/H9A -OH	Ac-ICVDhtQDWGAHRCT-COOH	19	5xmore
Ac-V4(Bpa)/H9A-OH	Ac-ICV(Bpa)QDWGAHRCT-COOH	20	49xmore
Ac-V4(Bpa)/H9A	Ac-ICV(Bpa)QDWGAHRCT-CONH ₂	21	86xmore
Ac-V4(Bta)/H9A -OH	Ac-ICV(Bta)PDWGAHRCT-COOH	22	65xmore
Ac-V4(Bta)/H9A	Ac-ICV(Bta)QDWGAHRCT-CONH ₂	23	64xmore
Ac-V4W/H9(2-Abu)	Ac-ICVWQDWG(2-Abu)HRCT-CONH ₂	24	64xmore
+G/V4W/H9A +AN -OH	<i>H</i> -GICVWQDWGAHRCTAN-COOH	25	38xmore
Ac-V4(5fW)/H9A	Ac-ICV(5fW)QDWGAHRCT- CONH ₂	26	31xmore
Ac-V4(5-MeW)/H9A	Ac-ICV(5-methyl-W)QDWGAHRCT- CONH ₂	27	67xmore
Ac-V4(1-MeW)/H9A	Ac-ICV(1-methyl-W)QDWGAHRCT-CONH ₂	28	264xmore
Ac-V4W/W7(5fW)/H9A	Ac-ICVWQD(5fW)GAHRCT-CONH ₂	29	121xmore
Ac-V4(5fW)/W7(5fW) /H9A	Ac-ICV(5fW)QD(5fW)GAHRCT- CONH ₂	30	NA

(continued)

Peptide	Sequence	SEQ ID NO:	Activity over compstatin
Ac-V4(5-MeW)/W7 (5fW)/H9A	Ac-ICV(<u>5-methyl-W</u>)QD(<u>5fW</u>)GAHRCT-CONH ₂	31	NA
Ac-V4(1 MeW)/W7 (5fW)/H9A	Ac-ICV(<u>1-methyl-W</u>)QD(<u>5fW</u>)GAHRCT-CONH ₂	32	264xmore
+G/V4(6fW)/W7(6fW) H9A+N-OH	H-GICV(<u>6fW</u>)QD(6fW)GAHRCTN-COOH	33	126xmore
Ac-V4(1-formyl-W) /H9A	Ac-ICV(<u>1-formyl-W</u>)QDWGAHRCT-CONH ₂	34	264xmore
Ac-V4(5-methoxy-W) /H9A	Ac-ICV(<u>1-methoxy-W</u>)QDWGAHRCT-CONH ₂	35	76xmore
G/V4(5f-W)/W7(fW) /H9A+N-OH	H-GICV(<u>5fW</u>)QD(<u>5fW</u>)GAHRCTN-COOH	36	112xmore
NA = not available			

[0073] Disclosed compstatin analogs may have a sequence selected from sequences 9-36; e.g. a sequence selected from SEQ ID NOs: 14, 21, 28, 29, 32, 33, 34, and 36; or a sequence selected from SEQ ID NOs: 30 and 31; or a sequence of SEQ ID NO: 28; or a sequence of SEQ ID NO: 32; or a sequence of SEQ ID NO: 34; or a sequence of SEQ ID NO: 36.

[0074] A disclosed blocking moiety B¹ may comprise an amino acid, which may be represented as XaaO. A disclosed blocking moiety B² may comprise an amino acid, which may be represented as XaaN. B¹ and/or B² may comprise a non-standard amino acid, such as a D-amino acid, N-alkyl amino acid (e.g., N-methyl amino acid). B¹ and/or B² may comprise a non-standard amino acid that is an analog of a standard amino acid. An amino acid analog may comprise a lower alkyl, lower alkoxy, or halogen substituent, as compared with a standard amino acid of which it is an analog. A substituent may be on a side chain or on an alpha carbon atom. A blocking moiety B¹ comprising an amino acid, e.g., a non-standard amino acid, may further comprise a moiety B^{1a}. For example, a blocking moiety B¹ may be represented as B^{1a}-XaaO. B^{1a} may neutralize or reduce a positive charge that may otherwise be present at the N-terminus at physiological pH. B^{1a} may comprise or consist of, e.g., an acyl group that, e.g., comprises between 1 and 12 carbons, e.g., between 1 and 6 carbons. B^{1a} may be selected from the group consisting of: formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, etc. A blocking moiety B² comprising an amino acid, e.g., a non-standard amino acid, may further comprise a moiety B^{2a}. For example, a blocking moiety B² may be represented as XaaN-B^{2a}, where N represents the appropriate number for the amino acid (which will depend on the numbering used in the rest of the peptide). B^{2a} may neutralize or reduce a negative charge that may otherwise be present at the C-terminus at physiological pH. B^{2a} may comprise or consist of a primary or secondary amine (e.g., NH₂). A blocking activity of moiety B^{1a}-XaaO and/or XaaN-B^{2a} may be provided by either or both components of the moiety. A blocking moiety or portion thereof, e.g., an amino acid residue, may contribute to increasing affinity of the compound for C3 or C3b and/or improve the activity of the compound. A contribution to affinity or activity of an amino acid residue may be at least as important as a contribution to blocking activity. For example, XaaO and/or XaaN in B^{1a}-XaaO and/or XaaN-B^{2a} may function mainly to increase affinity or activity of the compound, while B^{1a} and/or B^{2a} may inhibit digestion of and/or neutralize a charge of the peptide. A disclosed compstatin analog may comprise the amino acid sequence of any of SEQ ID NOs: 5-36, wherein SEQ ID NOs: 5-36 is further extended at the N- and/or C-terminus. Such a sequence may be represented as B^{1a}-XaaO -SEQUENCE - XaaN-B^{2a}, where SEQUENCE represents any of SEQ ID NOs: 5-36, wherein B^{1a} and B^{2a} may independently be present or absent. For example, a disclosed compstatin analog comprises B^{1a}-XaaO - X'aa1 - X'aa2 - X'aa3 - X'aa4 -Gln-Asp-Xaa-Gly- X"aa1- X"aa2- X"aa3- X"aa4-X"aa5 - XaaN-B^{2a} (SEQ ID NO: 37A), where X'aa1 - X'aa2 - X'aa3 - X'aa4, Xaa, X"aa1, X"aa2, X"aa3, X"aa4, and X"aa5 are as set forth above for SEQ ID NO: 5.

[0075] A disclosed compstatin analog comprises B^{1a}-XaaO - Xaa1 - Cys - Val -Xaa2 - Gln - Asp - Xaa2*-Gly - Xaa3 -His -Arg - Cys-Xaa4 - XaaN-B^{2a} (SEQ ID NO: 38A), where Xaa1, Xaa2, Xaa2*, Xaa3, and Xaa4 are as set forth above for SEQ ID NO: 6 or wherein Xaa1, Xaa2, Xaa2*, Xaa3, and Xaa4 are as set forth for SEQ ID NO: 6 or SEQ ID NO: 7.

[0076] A disclosed compstatin analog comprises B^{1a}-XaaO - Xaa1 - Xaa2- Xaa3 - Xaa4 - Xaa5 - Xaa6 - Xaa7 - Xaa8 - Xaa9 - Xaa10- Xaa11- Xaa12-Xaa13-XaaN-B^{2a} (SEQ ID NO: 39A) wherein Xaa1, Xaa2, Xaa3, Xaa4, Xaa5, Xaa6, Xaa7, Xaa8, Xaa9, Xaa10, Xaa11, Xaa12, and Xaa13 are identical to amino acids at positions 1-13 of any of SEQ ID NOs: 9-36.

[0077] XaaO and/or XaaN in disclosed compstatin analog sequences may comprise an amino acid that comprises an aromatic ring having an alkyl substituent at one or more positions. An alkyl substituent may be a lower alkyl substituent such as a methyl or ethyl group. Such a substituent may be located at any position that does not destroy the aromatic character of the compound or the aromatic character of a ring to which the substituent is attached. Such a substituent may be located at position 1, 2, 3, 4, or 5. In some disclosed aspects XaaO may comprise an O-methyl analog of tyrosine, 2-hydroxyphenylalanine or 3-hydroxyphenylalanine. For purposes of the present disclosure, a lower case "m" followed by a three letter amino acid abbreviation may be used to specifically indicate that an amino acid is an N-methyl amino acid. For example, where the abbreviation "mGly" appears herein, it denotes N-methyl glycine (also sometimes referred to as sarcosine or Sar). In some disclosed aspects, XaaO may be or comprise mGly, Tyr, Phe, Arg, Trp, Thr, Tyr(Me), Cha, mPhe, mVal, mIle, mAla, DTyr, DPhe, DArg, DTrp, DThr, DTyr(Me), mPhe, mVal, mIle, DAla, or DCha. For example, a disclosed compstatin analog comprises a peptide having a sequence B¹-Ile-[Cys-Val-Trp(Me)-Gln-Asp-Trp-mGly-Ala-His-Arg-Cys]-mIle-B² (SEQ ID NO: 40A) or B¹-Ile-[Cys-Val-Trp(Me)-Gln-Asp-Trp-mGly-Ala-His-Arg-Cys]-mIle-B² (SEQ ID NO: 41A). The two Cys residues are joined by a disulfide bond in the active compounds. The peptide may be acetylated at the N-terminus and/or amidated at the C-terminus. B¹ may comprise B^{1a}-XaaO and/or B² may comprise XaaN-B^{2a}, as disclosed above. B¹ may comprise or consist of Gly, mGly, Tyr, Phe, Arg, Trp, Thr, Tyr(Me), mPhe, mVal, mIle, mAla, DTyr, DPhe, DTrp, DCha, DAla and B² may comprise NH₂, e.g., a carboxy terminal -OH of mIle may be replaced by NH₂. B¹ may comprise or consist of mGly, Tyr, DTyr, or Tyr(Me) and B² may comprise NH₂, e.g., a carboxy terminal -OH of mIle may be replaced by NH₂. An Ile at position Xaa1 may be replaced by Gly.

[0078] Complement inhibition potency and/or C3b binding parameters of selected compstatin analogs are described in WO/2010/127336 (PCT/US2010/033345) and/or in Qu, et al., Immunobiology (2012), doi:10.1016/j.imbio.2012.06.003.

[0079] A blocking moiety or portion thereof, e.g., an amino acid residue, may contribute to increasing affinity of the compound for C3 or C3b and/or improve the activity of the compound. A contribution to affinity or activity of an amino acid or amino acid analog may be more significant than a blocking activity.

[0080] Certain disclosed compstatin analogs have a sequence as set forth in Table 1, but where the Ac- group is replaced by an alternate blocking moiety B¹. The -NH₂ group may be replaced by an alternate blocking moiety B².

[0081] The compstatin analog of the invention may bind to substantially the same region of the β chain of human C3 as does compstatin. The compstatin analog of the invention may bind to a fragment of the C-terminal portion of the β chain of human C3 having a molecular weight of about 40 kDa to which compstatin binds (Soulika, A.M., et al., Mol. Immunol., 35:160, 1998; Soulika, A.M., et al., Mol. Immunol. 43(12):2023-9, 2006). The compstatin analog of the invention may bind to the binding site of compstatin as determined in a compstatin-C3 structure, e.g., a crystal structure or NMR-derived 3D structure. The compstatin analog may substitute for compstatin in a compstatin-C3 structure and form substantially the same intermolecular contacts with C3 as compstatin. The compstatin analog may bind to the binding site of a peptide having a sequence set forth in Table 1, e.g., SEQ ID NO: 14, 21, 28, 29, 32, 33, 34, or 36, 37, 37A, 38A, 39A, 40A, or 41A or another compstatin analog sequence disclosed herein in a peptide-C3 structure, e.g., a crystal structure. The compstatin analog may bind to the binding site of a peptide having SEQ ID NO: 30 or 31 in a peptide-C3 structure, e.g., a crystal structure. The compstatin analog may substitute for the peptide of SEQ ID NO: 9-36, e.g., may substitute for the peptide of SEQ ID NO: 14, 21, 28, 29, 32, 33, 34, or 36, 37, 37A, 38A, 39A, 40A, or 41A or another compstatin analog sequence disclosed herein in a peptide-C3 structure and form substantially the same intermolecular contacts with C3 as the peptide. The compstatin analog may substitute for the peptide of SEQ ID NO: 30 or 31 in a peptide-C3 structure and form substantially the same intermolecular contacts with C3 as the peptide.

[0082] One of ordinary skill in the art will readily be able to determine whether a compstatin analog binds to a fragment of the C-terminal portion of the β chain of C3 using routine experimental methods. For example, one of skill in the art could synthesize a photocrosslinkable version of the compstatin analog by including a photo-crosslinking amino acid such as *p*-benzoyl-L-phenylalanine (Bpa) in the compound, e.g., at the C-terminus of the sequence (Soulika, A.M., et al, *supra*). Optionally additional amino acids, e.g., an epitope tag such as a FLAG tag or an HA tag could be included to facilitate detection of the compound, e.g., by Western blotting. The compstatin analog is incubated with the fragment and crosslinking is initiated. Colocalization of the compstatin analog and the C3 fragment indicates binding. Surface plasmon resonance may also be used to determine whether a compstatin analog binds to the compstatin binding site on C3 or a fragment thereof. One of skill in the art would be able to use molecular modeling software programs to predict whether a compound would form substantially the same intermolecular contacts with C3 as would compstatin or a peptide having the sequence of any of the peptides in Table 1, e.g., SEQ ID NO: 14, 21, 28, 29, 32, 33, 34, or 36, or SEQ ID NO: 30 or 31, 37, 37A, 38A, 39A, 40A, or 41A or another compstatin analog sequence disclosed herein.

[0083] Compstatin analogs may be prepared by various synthetic methods of peptide synthesis known in the art *via* condensation of amino acid residues, e.g., in accordance with conventional peptide synthesis methods, or may be prepared by expression *in vitro* or in living cells from appropriate nucleic acid sequences encoding them using methods known in the art. For example, peptides may be synthesized using standard solid-phase methodologies as described in Malik, *supra*, Katragadda, *supra*, WO2004026328, and/or WO2007062249. Potentially reactive moieties such as amino and carboxyl groups, reactive functional groups, etc., may be protected and subsequently deprotected using

various protecting groups and methodologies known in the art. See, e.g., "Protective Groups in Organic Synthesis", 3rd ed. Greene, T. W. and Wuts, P. G., Eds., John Wiley & Sons, New York: 1999. Peptides may be purified using standard approaches such as reversed-phase HPLC. Separation of diastereomeric peptides, if desired, may be performed using known methods such as reversed-phase HPLC. Preparations may be lyophilized, if desired, and subsequently dissolved in a suitable solvent, e.g., water. The pH of the resulting solution may be adjusted, e.g. to physiological pH, using a base such as NaOH. Peptide preparations may be characterized by mass spectrometry if desired, e.g., to confirm mass and/or disulfide bond formation. See, e.g., Mallik, 2005, and Katragadda, 2006.

[0084] In the invention, the compstatin analog comprises a molecule which is polyethylene glycol (PEG), to stabilize the compound, reduce its immunogenicity, increase its lifetime in the body, increase or decrease its solubility, and/or increase its resistance to degradation. In the invention, the compstatin analog comprises a linear PEG moiety having a molecular weight of 40 kD and coupled to each of two compstatin analog moieties as described in more detail herein. Methods for pegylation are well known in the art (Veronese, F.M. & Harris, Adv. Drug Deliv. Rev. 54, 453-456, 2002; Davis, F.F., Adv. Drug Deliv. Rev. 54, 457-458, 2002); Hinds, K.D. & Kim, S.W. Adv. Drug Deliv. Rev. 54, 505-530 (2002; Roberts, M.J., Bentley, M.D. & Harris, J.M. Adv. Drug Deliv. Rev. 54, 459-476; 2002); Wang, Y.S. et al. Adv. Drug Deliv. Rev. 54, 547-570, 2002). A wide variety of polymers such as PEGs and modified PEGs, including derivatized PEGs to which polypeptides can conveniently be attached are described in Nektar Advanced Pegylation 2005-2006 Product Catalog, Nektar Therapeutics, San Carlos, CA, which also provides details of appropriate conjugation procedures.

[0085] In the invention, the compstatin analog comprises two compstatin analog moieties coupled to the linear PEG moiety having a molecular weight of 40 kD via an amide bond, as described in more detail herein. The compstatin analog moieties are thus covalently linked to the polymer. The compstatin analog moieties are identical. Thus, the compstatin analog of the invention comprises two instances, or copies, of a single compstatin analog moiety as defined herein. In the invention the number of compstatin analog moieties ("n") is 2.

[0086] In the invention, the compstatin analog moiety comprises a Lys residue. The Lys residue is separated from the cyclic portion of the compstatin analog moiety by an AEEAc spacer as defined herein.

[0087] Any of a variety of polymeric backbones or scaffolds can be used in other disclosed compstatin analogs. For example, disclosed polymeric backbones or scaffolds may be a polyamide, polysaccharide, polyanhydride, polyacrylamide, polymethacrylate, polypeptide, polyethylene oxide, or dendrimer. Suitable methods and polymeric backbones are described, e.g., in WO98/46270 (PCT/US98/07171) or WO98/47002 (PCT/US98/06963). Disclosed polymeric backbones or scaffolds can comprise multiple reactive functional groups, such as carboxylic acids, anhydride, or succinimide groups. The polymeric backbone or scaffold may be reacted with the compstatin analogs. A disclosed compstatin analog may comprise any of a number of different reactive functional groups, such as carboxylic acids, anhydride, or succinimide groups, which are reacted with appropriate groups on the polymeric backbone. Alternately, monomeric units that could be joined to one another to form a polymeric backbone or scaffold can be first reacted with the compstatin analogs and the resulting monomers can be polymerized. Short chains may be prepolymerized, functionalized, and then a mixture of short chains of different composition may be assembled into longer polymers.

V. Compstatin Mimetics

[0088] The structure of compstatin is known in the art, and NMR structures for a number of compstatin analogs having higher activity than compstatin are also known (Malik, *supra*). Structural information may be used to design compstatin mimetics.

[0089] Disclosed herein is a compstatin mimetic which is any compound that competes with compstatin or any compstatin analog (e.g., a compstatin analog whose sequence is set forth in Table 1) for binding to C3 or a fragment thereof (such as a 40 kD fragment of the β chain to which compstatin binds). A disclosed compstatin mimetic may have an activity equal to or greater than that of compstatin. A disclosed compstatin mimetic may be more stable, orally available, or has a better bioavailability than compstatin. A compstatin mimetic may be a peptide, nucleic acid, or small molecule. A disclosed compstatin mimetic may be a compound that binds to the binding site of compstatin as determined in a compstatin-C3 structure, e.g., a crystal structure or a 3-D structure derived from NMR experiments. A disclosed compstatin mimetic may be a compound that could substitute for compstatin in a compstatin-C3 structure and would form substantially the same intermolecular contacts with C3 as compstatin. A disclosed compstatin mimetic may be a compound that binds to the binding site of a peptide having a sequence set forth in Table 1, e.g., SEQ ID NO: 14, 21, 28, 29, 32, 33, 34, or 36 or, for example, SEQ ID NO: 30 or 31 or other compstatin analog sequence, in a peptide-C3 structure. A disclosed compstatin mimetic may be a compound that could substitute for a peptide having a sequence set forth in Table 1, e.g., SEQ ID NO: 14, 21, 28, 29, 32, 33, 34, or 36 or, for example, SEQ ID NO: 30 or 31 or other compstatin analog sequence, in a peptide-C3 structure and would form substantially the same intermolecular contacts with C3 as the peptide. A disclosed compstatin mimetic may have a non-peptide backbone but have side chains arranged in a sequence designed based on the sequence of compstatin.

[0090] One of skill in the art will appreciate that once a particular desired conformation of a short peptide has been

ascertained, methods for designing a peptide or peptidomimetic to fit that conformation are well known. See, e.g., G.R. Marshall (1993), *Tetrahedron*, 49: 3547-3558; Hruby and Nikiforovich (1991), in *Molecular Conformation and Biological Interactions*, P. Balaram & S. Ramasehan, eds., Indian Acad. of Sci., Bangalore, PP. 429-455), Eguchi M, Kahn M., *Mini Rev Med Chem.*, 2(5):447-62, 2002. The design of peptide analogs may be further refined by considering the contribution of various side chains of amino acid residues, e.g., for the effect of functional groups or for steric considerations as described in the art for compstatin and analogs thereof, among others.

[0091] It will be appreciated by those of skill in the art that a peptide mimic may serve equally well as a peptide for the purpose of providing the specific backbone conformation and side chain functionalities required for binding to C3 and inhibiting complement activation. Accordingly, disclosed herein is the concept to produce and utilize C3-binding, complement-inhibiting compounds through the use of either naturally-occurring amino acids, amino acid derivatives, analogs or non-amino acid molecules capable of being joined to form the appropriate backbone conformation. A non-peptide analog, or an analog comprising peptide and non-peptide components, is sometimes referred to herein as a "peptidomimetic" or "isosteric mimetic," to designate substitutions or derivations of a peptide that possesses much the same backbone conformational features and/or other functionalities, so as to be sufficiently similar to the exemplified peptides to inhibit complement activation. More generally, a compstatin mimetic is any compound that would position pharmacophores similarly to their positioning in compstatin, even if the backbone differs.

[0092] The use of peptidomimetics for the development of high-affinity peptide analogs is well known in the art. Assuming rotational constraints similar to those of amino acid residues within a peptide, analogs comprising non-amino acid moieties may be analyzed, and their conformational motifs verified, by means of the Ramachandran plot (Hruby & Nikiforovich 1991), among other known techniques.

[0093] One of skill in the art will readily be able to establish suitable screening assays to identify additional compstatin mimetics and to select those having desired inhibitory activities. For example, compstatin or an analog thereof could be labeled (e.g., with a radioactive or fluorescent label) and contacted with C3 in the presence of different concentrations of a test compound. The ability of the test compound to diminish binding of the compstatin analog to C3 is evaluated. A test compound that significantly diminishes binding of the compstatin analog to C3 is a candidate compstatin mimetic. For example, a test compound that diminishes steady-state concentration of a compstatin analog-C3 complex, or that diminishes the rate of formation of a compstatin analog-C3 complex by at least 25%, or by at least 50%, is a candidate compstatin mimetic. One of skill in the art will recognize that a number of variations of this screening assay may be employed. Compounds to be screened include natural products, libraries of aptamers, phage display libraries, compound libraries synthesized using combinatorial chemistry, etc. Disclosed herein is the synthesizing of a combinatorial library of compounds based upon the core sequence described above and screening the library to identify compstatin mimetics. Any of these methods could also be used to identify new compstatin analogs having higher inhibitory activity than compstatin analogs tested thus far.

VI. Cell-reactive or Long-Acting Compstatin Analogs

[0094] Also disclosed herein are cell-reactive compstatin analogs. A cell-reactive compstatin analog may comprise a compound of formula A-L-M, wherein A is a moiety that comprises a cell-reactive functional group J, L is an optionally present linking portion, and M comprises a compstatin analog moiety. Such a disclosed compstatin analog moiety can comprise any compstatin analog, e.g., any compstatin analog described above. Formula A-L-M encompasses variants in which A-L is present at the N-terminus of the compstatin analog moiety, variants in which A-L is present at the C-terminus of the compstatin analog moiety, variants in which A-L is attached to a side chain of an amino acid of the compstatin analog moiety, and variants where the same or different A-Ls are present at both ends of M. In disclosed compounds of formula A-L-M, a functional group of the compstatin analog will have reacted with a functional group of L to form a covalent bond to A or L. For example, a disclosed cell-reactive compstatin analog in which the compstatin analog moiety comprises a compstatin analog that contains an amino acid with a side chain containing a primary amine (NH₂) group (which compstatin analog can be represented by formula R¹- (NH₂)), can have a formula R¹-NH-L- A in which a new covalent bond to L (e.g., N-C) has been formed and a hydrogen lost. Thus the term "compstatin analog moiety" includes molecular structures in which at least one atom of a compstatin analog participates in a covalent bond with a second moiety, e.g., via modification of a side chain. Similar considerations apply to compstatin analog moieties present in multivalent compounds as described above. Disclosed herein is a blocking moiety at the N-terminus or C-terminus of a compstatin analog, e.g., a compstatin analog described in Section IV above, replaced by A-L in the structure of a cell-reactive compstatin analog. In disclosed aspects, A or L may comprise a blocking moiety. A disclosed cell-reactive compstatin analog may have a molar activity of at least about 10%, 20%, or 30%, e.g., between 30% and 40%, between 30% and 50%, between 30% and 60%, between 30% and 70%, between 30% and 80%, between 30% and 90%, or more, of the activity of a corresponding compstatin analog having the same amino acid sequence (and, if applicable, one or more blocking moiety(ies)) but not comprising a cell-reactive moiety. Disclosed cell-reactive compstatin analogs may comprise multiple compstatin analog moieties, and the molar activity of the cell-reactive compstatin analog

may be at least about 10%, 20%, or 30%, e.g., between 30% and 40%, between 30% and 50%, between 30% and 60%, between 30% and 70%, between 30% and 80%, between 30% and 90%, or more, of the sum of the activities of said compstatin analog moieties.

[0095] In disclosed aspects, cell-reactive moiety A can comprise any of a variety of different cell-reactive functional groups J. In general, a cell-reactive functional group may be selected based at least in part on factors such as (a) the particular functional group to be targeted; (b) the ability of the reactive functional group to react with the target functional group under physiologically acceptable ex vivo conditions (e.g., physiologically acceptable pH and osmolarity) and/or in vivo conditions (e.g., in blood); (c) the specificity of the reaction between the reactive functional group and the target functional group under physiologically acceptable ex vivo conditions and/or in vivo; (d) the stability (e.g., under in vivo conditions) of the covalent bond that would result from reaction of the reactive functional group with its target functional group; (e) the ease of synthesizing a cell-reactive compstatin analog comprising the reactive functional group, etc. A reactive functional group that reacts with its target chemical group without releasing a leaving group may be selected. A reactive functional group that results in release of a leaving group upon reaction with a target may be selected. Compounds containing such groups may be useful, e.g., to monitor progress and/or extent of a reaction. A leaving group may be physiologically acceptable to cells, tissues, or organs in the amount generated (e.g., based on concentration and/or absolute amount generated) and/or is medically acceptable to a subject in the amount generated in vivo (e.g., based on concentration in a relevant body fluid such as blood and/or based on the absolute amount generated). A leaving group generated ex vivo may be at least in part removed, e.g., by washing cells or by washing or perfusing a tissue or organ, e.g., with saline.

[0096] A disclosed cell-reactive functional group may react with a side chain of an amino acid residue and/or with an N-terminal amino group or C-terminal carboxyl group of a protein. The cell-reactive functional group may be reactive with sulfhydryl (-SH) groups, which are found in the side chains of cysteine residues. A maleimide group may be used. Maleimide groups react with sulfhydryl groups of cysteine residues of proteins at physiologic pH and form a stable thioether linkage. A haloacetyl group, such as an iodoacetyl or a bromoacetyl group, may be used. Haloacetyls react with sulfhydryl groups at physiologic pH. The reaction of the iodoacetyl group proceeds by nucleophilic substitution of iodine with a sulfur atom from a sulfhydryl group resulting in a stable thioether linkage. An iodoacetamide group may be used. The cell-reactive functional group may react with amino (-NH₂) groups, which are present at the N-termini of proteins and in the side chain of lysine residues (ϵ -amino group). An activated ester, e.g., a succinimidyl ester (i.e., NHS ester) may be used. For example, N-hydroxysuccinimide (NHS) or its water-soluble analog (sulfo-NHS) can be used in the synthesis, whereby the resulting cell-reactive compstatin analog comprises an NHS ester. A cell-reactive functional group may react with carboxyl (-COOH) groups, which are present at the C-termini of proteins and in the side chains of various amino acid residues. The cell-reactive compstatin analog may be reactive with hydroxyl (-OH) groups, which are present in the side chains of various amino acids and in carbohydrate moieties of glycosylated proteins.

[0097] In disclosed aspects, linking portion L can comprise any one or more aliphatic and/or aromatic moieties consistent with the formation of a stable compound joining the linked moieties. The term "stable", as used herein, preferably refers to compounds which possess stability sufficient to allow manufacture and which maintain the integrity of the compound for a sufficient period of time, e.g., to be useful for one or more purposes described herein. In disclosed aspects, L may comprise a saturated or unsaturated, substituted or unsubstituted, branched or unbranched, aliphatic chain having a length of between 1 and 30, between 1 and 20, between 1 and 10, between 1 and 6, or 5 or less carbon atoms, where length refers to the number of C atoms in the main (longest) chain. The aliphatic chain may comprise one or more heteroatoms (O, N, S), which may be independently selected. At least 50% of the atoms in the main chain of L may be carbon atoms. L may comprise a saturated alkyl moiety (CH₂)_n, wherein n is between 1 and 30.

[0098] In some disclosed aspects, L may comprise one or more heteroatoms and have a length of between 1 and 1000, between 1 and 800, between 1 and 600, between 1 and 400, between 1 and 300, between 1 and 200, between 1 and 100, between 1 and 50, between 1 and 30, or between 1 and 10 total carbon atoms in a chain. L may comprise an oligo(ethylene glycol) moiety $-(O-CH_2-CH_2-)_n$ wherein n is between 1 and 500, between 1 and 400, between 1 and 300, between 1 and 200, between 1 and 100, between 10 and 200, between 200 and 300, between 100 and 200, between 40 and 500, between 30 and 500, between 20 and 500, between 10 and 500, between 1 and 40, between 1 and 30, between 1 and 20, or between 1 and 10.

[0099] In some disclosed aspects, L may comprise an unsaturated moiety such as -CH=CH- or -CH₂-CH=CH-; a moiety comprising a non-aromatic cyclic ring system (e.g., a cyclohexyl moiety), an aromatic moiety (e.g., an aromatic cyclic ring system such as a phenyl moiety); an ether moiety (-C-O-C-); an amide moiety (-C(=O)-N-); an ester moiety (-CO-O-); a carbonyl moiety (-C(=O)-); an imine moiety (-C=N-); a thioether moiety (-C-S-C-); an amino acid residue; and/or any moiety that can be formed by the reaction of two compatible reactive functional groups. One or more moieties of a linking portion or cell-reactive moiety may be substituted by independent replacement of one or more of the hydrogen (or other) atoms thereon with one or more moieties including, but not limited to aliphatic; aromatic, aryl; alkyl, aralkyl, alkanoyl, aroyl, alkoxy; thio; F; Cl; Br; I; -NO₂; -CN; -CF₃; -CH₂CF₃; -CHC12; -CH₂OH; -CH₂CH₂OH; -CH₂NH₂; -CH₂SO₂CH₃; - or -GRG1 wherein G is -O-, -S-, -NRG₂-, -C(=O)-, -S(=O)-, -SO₂-, -C(=O)O-, -C(=O)NRG₂-, -OC(=O)-,

-NRG2C(=O)-, -OC(=O)O-, -OC(=O)NRG2-, -NRG2C(=O)O-, -NRG2C(=O)NRG2-, -C(=S)-, -C(=S)S-, -SC(=S)-, -SC(=S)S-, -C(=NRG2)-, -C(=NRG2)O-, -C(=NRG2)NRG3-, -OC(=NRG2)-, -NRG2C(=NRG3)-, -NRG2SO2-, -NRG2SO2NRG3-, or -SO2NRG2-, wherein each occurrence of RG1, RG2 and RG3 independently includes, but is not limited to, hydrogen, halogen, or an optionally substituted aliphatic, aromatic, or aryl moiety. It will be appreciated that cyclic ring systems when present as substituents may optionally be attached via a linear moiety.

[0100] In some disclosed aspects, L can comprise one or more of any of the moieties described in the preceding paragraph. L may comprise two or more different moieties linked to one another to form a structure typically having a length of between 1 to about 60 atoms, between 1 to about 50 atoms, e.g., between 1 and 40, between 1 and 30, between 1 and 20, between 1 and 10, or between 1 and 6 atoms, where length refers to the number of atoms in the main (longest) chain. L may comprise two or more different moieties linked to one another to form a structure typically having between 1 to about 40, e.g., between 1 and 30, e.g., between 1 and 20, between 1 and 10, or between 1 and 6 carbon atoms in the main (longest) chain. In general, the structure of such a cell-reactive compstatin analog can be represented by formula A-(L^{Pj})_j-M, wherein j is typically between 1 and 10, and each L^{Pj} is independently selected from among the moieties described in the preceding paragraph. L may comprise one or more carbon-containing chains such as -(CH₂)_n- and/or -(O-CH₂-CH₂)_n, which are joined covalently to each other and/or to a cell-reactive functional group or compstatin analog, e.g., by moieties (e.g., amide, ester, or ether moieties) that result from the reaction of two compatible reactive functional groups. L may comprise an oligo(ethylene glycol) moiety and/or a saturated alkyl chain. L may comprise -(CH₂)_m-C(=O)-NH-(CH₂CH₂O)_n(CH₂)_pC(=O)- or -(CH₂)_m-C(=O)-NH-(CH₂)_p(OCH₂CH₂)_nC(=O)-. Typically, m, n, and p are selected so that the number of carbons in the chain is between 1 and 500, e.g., between 2 and 400, between 2 and 300, between 2 and 200, between 2 and 100, between 2 and 50, between 4 and 40, between 6 and 30, or between 8 and 20. Typically, m is between 2 and 10, n is between 1 and 500, and/or p is between 2 and 10. Typically, m is between 2 and 10, n is between 1 and 400, and/or p is between 2 and 10. Typically, m is between 2 and 10, n is between 1 and 300, and/or p is between 2 and 10. Typically, m is between 2 and 10, n is between 1 and 200, and/or p is between 2 and 10. Typically, m is between 2 and 10, n is between 1 and 100, and/or p is between 2 and 10. Typically, m is between 2 and 10, n is between 1 and 50, and/or p is between 2 and 10. Typically, m is between 2 and 10, n is between 1 and 25, and/or p is between 2 and 10. Typically, m is between 2 and 10, n is between 1 and 8, and/or p is between 2 and 10. Optionally, at least one -CH₂- is replaced by CH-R, wherein R can be any substituent. Optionally, at least one -CH₂- is replaced by a heteroatom, cyclic ring system, amide, ester, or ether moiety. Typically, L does not comprise an alkyl group having more than 3 carbon atoms in the longest chain. Typically, L does not comprise an alkyl group having more than 4, 5, 6, 7, 8, 9, 10, or 11 carbon atoms in the longest chain.

[0101] In some disclosed aspects, A comprises a cell-reactive functional group J and a linker L¹ comprising a linking portion L^{P1} and a reactive functional group that reacts with the compstatin analog to generate A-M. A bifunctional linker L² comprising two reactive functional groups and a linking portion L^{P2} may be used. The reactive functional groups of L may react with appropriate reactive functional groups of A and M to produce a cell-reactive compstatin analog A-L-M. The compstatin analog may comprise a linker L³ comprising a linking portion L^{P3}. For example, as discussed below, a linker comprising a reactive functional group may be present at the N- or C-terminus or a moiety comprising a reactive functional group may be attached to the N- or C-terminus via a linker. Thus L may contain multiple linking portions L^P contributed, e.g., by A, by linker(s) used to join A and M, and/or by the compstatin analog. It will be understood that, when present in the structure A-L-M, certain reactive functional group(s) present prior to reaction in L¹, L², L³, etc., will have undergone reaction, so that only a portion of said reactive functional group(s) will be present in the final structure A-L-M, and the compound will contain moieties formed by reaction of said functional groups. In general, if a compound contains two or more linking portions, the linking portions can be the same or different, and can be independently selected. Multiple linking portions L^P can be attached to one another to form a larger linking portion L, and at least some of such linking portions can have one or more compstatin analog(s) and/or cell-reactive functional group(s) attached thereto. The compstatin analogs can be the same or different and, if different, can be independently selected. The same applies to the linking portions and reactive functional groups. At least one linkage may be a stable non-covalent linkage such as a biotin/(strept)avidin linkage or other noncovalent linkage of approximately equivalent strength.

[0102] A disclosed cell-reactive compstatin analog comprises a compstatin analog in which any of SEQ ID NOs: 3-36, 37A, 38A, 39A, 40A, or 41A is extended by one or more amino acids at the N-terminus, C-terminus, or both, wherein at least one of the amino acids has a side chain that comprises a reactive functional group such as a primary or secondary amine, a sulfhydryl group, a carboxyl group (which may be present as a carboxylate group), a guanidino group, a phenol group, an indole ring, a thioether, or an imidazole ring. The amino acid(s) may be L-amino acids. Any one or more of the amino acid(s) may be a D-amino acid. If multiple amino acids are added, the amino acids can be independently selected. The reactive functional group (e.g., a primary or secondary amine) may be used as a target for addition of a moiety comprising a cell-reactive functional group. Amino acids having a side chain that comprises a primary or secondary amine include lysine (Lys) and diaminocarboxylic acids of general structure NH₂(CH₂)_nCH(NH₂)COOH such as 2,3-diaminopropionic acid (dapa), 2,4-diaminobutyric acid (daba), and ornithine (orn), wherein n = 1 (dapa), 2 (daba), and 3 (orn), respectively. At least one amino acid may be cysteine, aspartic acid, glutamic acid, arginine, tyrosine, tryptophan,

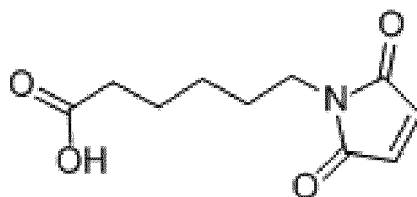
methionine, or histidine. Cysteine has a side chain comprising a sulfhydryl group. Aspartic acid and glutamic acid have a side chain comprising a carboxyl group (ionizable to a carboxylate group). Arginine has a side chain comprising a guanidino group. Tyrosine has a side chain comprising a phenol group (ionizable to a phenolate group). Tryptophan has a side chain comprising an indole ring include include, e.g., tryptophan. Methionine has a side chain comprising a thioether group include, e.g., methionine. Histidine has a side chain comprising an imidazole ring. A wide variety of non-standard amino acids having side chains that comprise one or more such reactive functional group(s) are available, including naturally occurring amino acids and amino acids not found in nature. See, e.g., Hughes, B. (ed.), *Amino Acids, Peptides and Proteins in Organic Chemistry, Volumes 1-4*, Wiley-VCH (2009-2011); Blaskovich, M., *Handbook on Syntheses of Amino Acids General Routes to Amino Acids*, Oxford University Press, 2010. One or more non-standard amino acid(s) may be used to provide a target for addition of a moiety comprising a cell-reactive functional group. Any one or more of the amino acid(s) may be protected as appropriate during synthesis of the compound. For example, one or more amino acid(s) may be protected during reaction(s) involving the target amino acid side chain. Wherein a sulfhydryl-containing amino acid is used as a target for addition of a moiety comprising a cell-reactive functional group, the sulfhydryl may be protected while the compound is being cyclized by formation of an intramolecular disulfide bond between other amino acids such as cysteines.

[0103] In the discussion in this paragraph, an amino acid having a side chain containing an amine group is disclosed as an example. Analogously, an amino acid having a side chain containing a different reactive functional group may be used. An amino acid having a side chain comprising a primary or secondary amine may be attached directly to the N-terminus or C-terminus of any of SEQ ID NOs: 3-36, 37, 37A, 38A, 39A, 40A, or 41A or via a peptide bond. An amino acid having a side chain comprising a primary or secondary amine may be attached to the N- or C-terminus of any of SEQ ID NOs: 3-36, 37, 37A, 38A, 39A, 40A, or 41A or via a linking portion, which may contain any one or more of the linking moieties described above. At least two amino acids may be appended to either or both termini. The two or more appended amino acids may be joined to each other by peptide bonds or at least some of the appended amino acids may be joined to each other by a linking portion, which may contain any one or more of the linking moieties described herein. Thus, a cell-reactive compstatin analog may comprise a compstatin analog moiety M of formula B1-R1-M₁-R2-B2, wherein M₁ represents any of SEQ ID NOs: 3-36, 37, 37A, 38A, 39A, 40A, or 41A, either R1 or R2 may be absent, at least one of R1 and R2 comprises an amino acid having a side chain that contains a primary or secondary amine, and B1 and B2 are optionally present blocking moieties. R1 and/or R2 may be joined to M₁ by a peptide bond or a non-peptide bond. R1 and/or R2 may comprise a linking portion L^{P3}. For example, R1 can have formula M₂-L^{P3} and/or R2 can have formula L^{P3}-M₂ wherein L^{P3} is a linking portion, and M₂ comprises at least one amino acid having a side chain comprising a primary or secondary amine. For example, M₂ can be Lys or an amino acid chain comprising Lys. L^{P3} may comprise or consist of one or more amino acids. For example, L^{P3} can be between 1 and about 20 amino acids in length, e.g., between 4 and 20 amino acids in length. L^{P3} may comprises or consist of multiple Gly, Ser, and/or Ala residues. Sometimes, L^{P3} does not comprise an amino acid that comprises a reactive SH group, such as Cys. Sometimes, L^{P3} comprises an oligo(ethylene glycol) moiety and/or a saturated alkyl chain. Sometimes, L^{P3} is attached to the N-terminal amino acid of M_i via an amide bond. Sometimes, L^{P3} is attached to the C-terminal amino acid of M_i via an amide bond. The compound may be further extended at either or both termini by addition of further linking portion(s) and/or amino acid(s). The amino acids can the same or different and, if different, can be independently selected. Two or more amino acids having side chains comprising reactive functional groups may be used, wherein the reactive functional groups can be the same or different. The two or more reactive functional groups can be used as targets for addition of two or more moieties. Sometimes, two or more cell-reactive moieties are added. Sometimes, a cell-reactive moiety and a targeting moiety are added. Sometimes, a linker and/or cell-reactive moiety is attached to an amino acid side chain after incorporation of the amino acid into a peptide chain. Sometimes, a linker and/or cell-reactive moiety is already attached to the amino acid side chain prior to use of the amino acid in the synthesis of a cell-reactive compstatin analog. For example, a Lys derivative having a linker attached to its side chain can be used. The linker may comprise a cell-reactive functional group or may subsequently be modified to comprise a cell-reactive functional group.

[0104] Certain cell-reactive compstatin analogs are described in further detail below. In the following discussion, a peptide having the amino acid sequence Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr (SEQ ID NO: 37) (corresponding to the compstatin analog of SEQ ID NO: 28, wherein asterisks in SEQ ID NO: 37 represent cysteines joined by a disulfide bond in the active compound, and (1Me)Trp represents 1-methyl-tryptophan)), is used as an exemplary compstatin analog moiety; maleimide (abbreviated Mal) is used as an example of a cell-reactive functional group; (CH₂)_n and (O-CH₂-CH₂)_n are used as examples of linking portions; lysine is used as an example of an amino acid comprising a reactive functional group (in some compounds), and acetylation and amidation of the N- and C-termini, respectively, are used as optionally present exemplary blocking moieties in some compounds and are represented in italics, i.e., as *Ac* and *NH₂* respectively. It will be appreciated that the compounds can be prepared using a variety of synthetic approaches and using a variety of precursors.

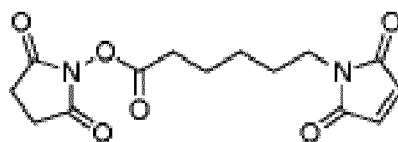
[0105] A disclosed cell-reactive moiety may be provided by a cell-reactive compound comprising a maleimide group (as a cell-reactive functional group) and an alkanolic acid (RCOOH), where R is an alkyl group. For example, 6-male-

meidocaproic acid ($\text{Mal}-(\text{CH}_2)_5\text{-COOH}$), depicted below, can be used.



Compound I

[0106] A disclosed cell-reactive moiety may be provided by a derivative of an alkanolic acid in which the carboxylic acid moiety has been activated, e.g., the OH moiety has been converted to a better leaving group. For example, the carboxyl group of compound I may be reacted with EDC, followed by reaction with NHS (which can optionally be provided as water-soluble sulfo-NHS), resulting in an N-hydroxysuccinimide ester derivative of 6-maleimidocaproic acid, i.e., 6-maleimidohexanoic acid N-hydroxysuccinimide (NHS) ester (depicted below).



Compound II

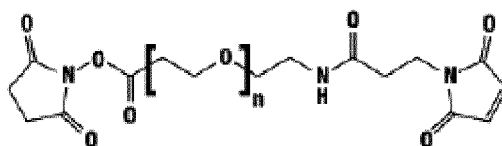
[0107] The compound of SEQ ID NO: 37 can be modified at the N- and/or C- terminus to generate a cell-reactive compstatin analog. For example, compound II can be used to generate the following cell-reactive compstatin analog by reaction with the N-terminal amino group of Ile.

[0108] Maleimide- $(\text{CH}_2)_5\text{-C(=O)-Ile-Cys}^*\text{-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys}^*\text{-Thr-NH}_2$ (SEQ ID NO: 38). It will be appreciated that in SEQ ID NO: 38 the -C(=O) moiety is attached to the immediately C-terminal amino acid (Ile), via a C-N bond, wherein the N is part of the amino acid and is not shown.

[0109] In other disclosed aspects, a maleimide group is linked to Thr at the C-terminus, resulting in the following cell-reactive compstatin analog:

[0110] $\text{Ac-Ile-Cys}^*\text{-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys}^*\text{-Thr-(C=O)-}(\text{CH}_2)_5\text{-maleimide}$ (SEQ ID NO: 39).

[0111] A cell-reactive compstatin analog can be synthesized using bifunctional linker (e.g., a heterobifunctional linker). An exemplary heterobifunctional linker comprising $(\text{CH}_2\text{-CH}_2\text{-O})_n$ and $(\text{CH}_2)_m$ (where $m=2$) moieties is shown below:



Compound III

[0112] Compound III comprises a maleimide group as a cell-reactive functional group and an NHS ester moiety that reacts readily with an amino group (e.g., an N-terminal amino group or an amino group of an amino acid side chain).

[0113] A version of compound III in which $n = 2$ can be used to generate the following cell-reactive compstatin analog using the compstatin analog of SEQ ID NO: 37:

[0114] Maleimide- $(\text{CH}_2)_2\text{-C(=O)-NH-CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{C(=O)-Ile-Cys}^*\text{-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys}^*\text{-Thr-NH}_2$ (SEQ ID NO: 40)

[0115] It will be appreciated that in the compound of SEQ ID NO: 40 a -C(=O) moiety is attached to the N-terminal amino acid (Ile residue via a C-N bond, wherein the N is part of the amino acid and is not shown). A linker may have the formula of Compound III wherein $n \geq 1$. Exemplary values for n in a $(\text{CH}_2\text{-CH}_2\text{-O})_n$ moiety are provided herein.

[0116] The alkyl chain that links the maleimide moiety to the rest of the molecule may contain more or fewer methylene units, the oligo(ethylene glycol) moiety may contain more or fewer ethylene glycol units, and/or there may be more or fewer methylene units flanking either or both sides of the oligo(ethylene glycol) moiety, as compared with the compound of SEQ ID NO: 39 or SEQ ID NO: 40. Some disclosed cell-reactive compstatin analogs illustrative of a few such variations are presented below (SEQ ID NOs: 41-46):

Maleimide-(CH₂)₂-C(=O)-NH-CH₂CH₂OCH₂CH₂C(=O)-Ile-Cys*-Val-(1Me)Trp - Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-NH₂ (SEQ ID NO: 41)

Maleimide-(CH₂)₃-C(=O)-NH-CH₂CH₂OCH₂CH₂OCH₂C(=O)-Ile-Cys*-Val-(1Me)Trp -Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-NH₂ (SEQ ID NO: 42)

Maleimide-(CH₂)₅-C(=O)-NH-CH₂CH₂OCH₂CH₂OCH₂C(=O)-Ile-Cys*-Val-(1Me)Trp -Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-NH₂ (SEQ ID NO: 43)

Maleimide-(CH₂)₄-C(=O)-NH-CH₂CH₂OCH₂CH₂OCH₂CH₂C(=O)-Ile-Cys*-Val-(1Me)Trp -Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-NH₂ (SEQ ID NO: 44)

Maleimide-(CH₂)₂-C(=O)-NH-CH₂CH₂OCH₂CH₂OCH₂CH₂C(=O)-Ile-Cys*-Val-(1Me)Trp -Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-NH₂ (SEQ ID NO: 45)

Maleimide-(CH₂)₅-C(=O)-NH-CH₂CH₂OCH₂CH₂OCH₂C(=O)-Ile-Cys*-Val-(1Me)Trp -Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-NH₂ (SEQ ID NO: 46)

SEQ ID NO: 37 may be extended to comprise a Lys residue at the N- or C-terminus of the peptide, e.g., as exemplified below for a C-terminal linkage:

Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-Lys-NH₂ (SEQ ID NO: 47).

A Lys residue may be attached to the N- or C- terminus of SEQ ID NO: 37 via a peptide linker, e.g., as exemplified below for a C-terminal linkage:

Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-(Gly)₅-Lys-NH₂ (SEQ ID NO: 48).

[0117] A linker comprising a primary or secondary amine may be added to the N- or C-terminus of a compstatin analog. The linker may comprise an alkyl chain and/or an oligo(ethylene glycol) moiety, such as NH₂(CH₂CH₂O)_nCH₂C(=O)OH (e.g., 8-amino-3,6-dioxaoctanoic acid (AEEAc) or 11-amino-3,6,9-trioxaundecanoic acid) or an NHS ester thereof (e.g., an NHS ester of 8-amino-3,6-dioxaoctanoic acid or 11-amino-3,6,9-trioxaundecanoic acid).

[0118] A disclosed compound is as follows (wherein the portion contributed by the linker is shown in bold):

NH₂(CH₂)₅C(=O)-Ile-Cys-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys-Thr-NH₂ (SEQ ID NO: 49)

NH₂(CH₂CH₂O)₂CH₂C(=O)-Ile-Cys-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys-Thr-NH₂ (SEQ ID NO: 50)

[0119] In some embodiments, a Lys residue is attached to the C-terminus of SEQ ID NO: 37 via a linker comprising a non-peptide portion. The linker comprises 8-AEEAc, resulting in the following compound (wherein the portion contributed by 8-AEEAc is shown in bold):

Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr -NH-**CH₂CH₂OCH₂CH₂OCH₂-C(=O)**-Lys-NH₂ (SEQ ID NO: 51)

[0120] It will be appreciated that in SEQ ID NOs: 49 and 50, a -C(=O) moiety is attached to the adjacent Ile residue via a C-N bond, wherein the N is part of the amino acid and is not shown. Similarly, in SEQ ID NO: 51, a -C(=O) moiety is attached to the adjacent Lys residue via a C-N bond, wherein the N is part of the amino acid and is not shown. It will also be appreciated that that in SEQ ID NO: 51 the NH moiety is attached to the immediately N-terminal amino acid (Thr), via a C-N bond, wherein the C is the carbonyl carbon of the amino acid and is not shown.

[0121] The compounds of SEQ ID NOs: 47-51 can readily be modified at the primary amine group to produce a cell-reactive compstatin analog. For example, the compounds of SEQ ID NOs: 47-51 (or other compounds comprising a primary or secondary amine and a compstatin analog moiety) can be reacted with 6-maleimidocaproic acid N-succinimidyl ester to produce the following cell-reactive compstatin analogs:

Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-Lys-(C(=O)-(CH₂)₅-Mal)-NH₂ (SEQ ID NO: 52).

Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-(Gly)₅-Lys--(C(=O)-(CH₂)₅-Mal)-NH₂ (SEQ ID NO: 53).

Mal-(CH₂)₅-(C(=O)-**NH(CH₂)₅C(=O)**)-Ile-Cys-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys-Thr-NH₂ (SEQ ID NO: 54)

Mal-(CH₂)₅-(C(=O)**NH(CH₂CH₂O)₂CH₂C(=O)**)-Ile-Cys-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys-Thr-NH₂ (SEQ ID NO: 55)

Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr -NH-**CH₂CH₂OCH₂CH₂OCH₂-C(=O)**-Lys-(C(=O)-(CH₂)₅-Mal)-NH₂ (SEQ ID NO: 56)

[0122] Another cell-reactive compstatin analog is represented as: $\text{Ac-Ile-Cys}^*-\text{Val}-(1\text{Me})\text{Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys}^*-\text{Thr-Lys-C}(=\text{O})-\text{CH}_2(\text{OCH}_2\text{CH}_2)_2\text{NH}(\text{C}(=\text{O})-(\text{CH}_2)_5\text{-Mal})-\text{NH}_2$ (SEQ ID NO: 57).

[0123] Also disclosed herein are variants of SEQ ID NOs: 38-57 in which -Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr- is replaced by an amino acid sequence comprising the amino acid sequence of any other compstatin analog, e.g., of any of SEQ ID NOs 3-27 or 29-36, 37, 37A, 38A, 39A, 40A, or 41A with the proviso that blocking moiety(ies) present at the N- and/or C-termini of a compstatin analog may be absent, replaced by a linker (which may comprise a blocking moiety), or attached to a different N- or C-terminal amino acid present in the corresponding variant(s).

[0124] Other disclosed bifunctional cross-linkers comprising a maleimide as a cell-reactive moiety and an NHS ester as an amine-reactive moiety include, e.g., succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB); succinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC); N- γ -maleimidobutryl-oxysuccinimide ester (GMBS). Addition of a sulfonate to the NHS ring results in water-soluble analogs such as sulfo-succinimidyl(4-iodoacetyl)-aminobenzoate (sulfo-SIAB), sulfo-succinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (sulfo-SMCC), sulfo-succinimidyl 4-(p-maleimidophenyl)butyrate (sulfo-SMPB), sulfo-N- γ -maleimidobutryl-oxysuccinimide ester (sulfo-GMBS) etc., which can avoid the need for an organic solvent. A long chain version of any of the foregoing, comprising a spacer arm between the NHS ester moiety and the remainder of the molecule, may be used. The spacer can comprise, e.g., an alkyl chain. An example is succinimidyl-4-[N-Maleimidomethyl]cyclohexane-1-carboxy-[6-amidocaproate].

[0125] A disclosed bifunctional linker comprises an NHS ester (as an amine-reactive moiety) and an iodoacetyl group (reactive with sulfhydryl groups). Such linkers include, e.g., N-succinimidyl(4-iodoacetyl)-aminobenzoate (SIAB); succinimidyl 6-[(iodoacetyl)-amino]hexanoate (SIAX); succinimidyl 6-[6-(((iodoacetyl)amino)-hexanoyl) amino]hexanoate (SIAXX); succinimidyl 4-(((iodoacetyl)amino)methyl)-cyclohexane-1-carboxylate (SIAC); succinimidyl 6-(((4-iodoacetyl)amino)methyl)-cyclohexane-1-carboxylate (SIACX);

[0126] Another disclosed bifunctional linker comprises an NHS ester (as an amine-reactive moiety) and a pyridyl disulfide group (as a cell-reactive moiety reactive with sulfhydryl groups). Examples include N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP); succinimidyl-oxycarbonyl- α -methyl- α -(2-pyridyldithio)toluene (SMPT) and versions comprising a sulfonate on the NHS ring and/or a spacer comprising an alkyl chain between the NHS ester moiety and the rest of the molecule (e.g., succinimidyl 6-[3-[2-pyridyldithio]-propionamido]hexanoate) (LC-SPDP). Variations of such linkers that include additional or different moieties could be used. For example, a longer or shorter alkyl chain could be used in a spacer, or an oligo(ethylene glycol) moiety instead of an alkyl chain.

[0127] In general, a cell-reactive compstatin analog can be synthesized using a variety of approaches. Cell-reactive compounds that comprise a cell-reactive functional group and a linker can often be purchased as preformed building blocks. For example, 6-maleimidocaproic acid and 6-maleimidocaproic acid N-hydroxysuccinimide ester can be purchased from various suppliers. Alternately, such compounds can be synthesized using methods known in the art. See, e.g., Keller O, Rudinger J. *Helv Chim Acta*. 58(2):531-41, 1975 and Hashida S, et al., *J Appl Biochem*, 6(1-2):56-63, 1984. See also, Hermanson, G. *supra*, and references therein, for discussion of methods and reagents of use for synthesizing conjugates. In general, the disclosure encompasses any method of producing a compound comprising a compstatin analog moiety and a cell-reactive functional group, and the resulting compounds.

[0128] An amino acid having a linker attached to a side chain may be used in the synthesis of a linear peptide. The linear peptide can be synthesized using standard methods for peptide synthesis known in the art, e.g., standard solid-phase peptide synthesis. The linear peptide is then cyclized (e.g., by oxidation of the Cys residues to form an intramolecular disulfide). The cyclic compound may then be reacted with a linker comprising a cell-reactive functional group. A moiety comprising a cell-reactive functional group may be reacted with a linear compound prior to cyclization thereof. In general, reactive functional groups can be appropriately protected to avoid undesired reaction with each other during synthesis of a cell-reactive compstatin analog. The cell-reactive functional group, any of the amino acid side chains, and/or either or both termini of the peptide may be protected during the reaction and subsequently deprotected. For example, SH groups of Cys residues and/or SH-reactive moieties such as maleimides can be protected until after cyclization to avoid reaction between them. The reaction conditions are selected based at least in part on the requirements of the particular reactive functional group(s) to achieve reasonable yield in a reasonable time period. Temperature, pH, and the concentration of the reagents can be adjusted to achieve the desired extent or rate of reaction. See, e.g., Hermanson, *supra*. The desired product can be purified, e.g., to remove unreacted compound comprising the cell-reactive functional group, unreacted compstatin analog, linker(s), products other than the desired cell-reactive compstatin analog that may have been generated in the reaction, other substances present in the reaction mixture, etc.

[0129] In the invention, a linker as described above is used in the production of the compstatin analog, which further comprises a linear polyethylene glycol (PEG) chain that e.g., stabilizes the compound, increases its lifetime in the body, increases its solubility, decreases its immunogenicity, and/or increases its resistance to degradation. Without limiting the invention in any way, such a moiety may be referred to herein as a "clearance reducing moiety" (CRM), and a compstatin analog comprising such a moiety may be referred to as a "long-acting compstatin analog". A long-acting compstatin analog may have an average plasma half-life of at least 1 day, e.g., 1 - 3 days, 3 - 7 days, 7 - 14 days, or 14 - 28 days, when administered IV at a dose of 10 mg/kg to humans or to non-human primates, or a dose of about 1-3

mg/kg, 3-5 mg/kg, 5-10 mg/kg, e.g., 7 mg/kg. A long-acting compstatin analog may have an average plasma half-life of at least 1 day, e.g., 1 - 3 days, 3 - 7 days, 7 - 14 days, or 14 - 28 days, when administered subcutaneously at, e.g., a dose of about 1-3 mg/kg, 3-5 mg/kg, 5 -10 mg/kg, e.g., 7 mg/kg to humans or to non-human primates. A long-acting compstatin analog may have an average plasma half-life (e.g., a terminal half-life) of between about 4-10, 5-9, 5-8, 6-9, 7-9, or 8-9 days, e.g., about 4,4.5, 5,5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 days when administered intravenously at, e.g., a dose of about 1-3 mg/kg, 3-5 mg/kg, or 5-10 mg/kg, e.g., 7 mg/kg to humans or to non-human primates. A long-acting compstatin analog may have an average plasma half-life (e.g., a terminal half-life) of between about 4-10, 5-9, 5-8, 6-9, 7-9, or 8-9 days, e.g., about 4,4.5, 5,5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 days, when administered subcutaneously at, e.g., a dose of about 1-3 mg/kg, 3-5 mg/kg, 5 -10 mg/kg, e.g., 7 mg/kg to humans or to non-human primates. A long-acting compstatin analog may be characterized in that it is extensively absorbed from the site of administration during the time period following subcutaneous injection and provides, e.g., at or after about 1-2 days following administration, a blood level comparable to that which would be achieved had the same amount of compound been administered intravenously instead. The blood level at or after about 2, 3, 4, 5, 6, 7, 8, or more days following administration of a subcutaneous dose may be within about 5%, 10%, 15%, 20%, or 25% of the blood level which would be achieved had the same amount of compound been administered intravenously instead. See, e.g., Figure 11, showing pharmacokinetics of an intravenously and subcutaneously administered dose of an exemplary compound described herein after about 1-2 days following administration. The average plasma half-life of a long-acting compstatin analog following administration IV at a dose of 10 mg/kg to humans or to non-human primates may be increased by at least a factor of 2, e.g., by a factor of 2-5, 5-10, 10-50, or 50-100 -fold or 100-150-fold or 150-200 fold as compared with that of a corresponding compstatin analog having the same amino acid sequence (and, if applicable, one or more blocking moiety(ies)) but not comprising the CRM. Such an increase in half-life may be observed following administration via other routes such as subcutaneous administration and/or using other doses, e.g., other doses described herein, e.g., 20 mg/kg. A compstatin analog of any of SEQ ID NOs: 3-36, 37, 37A, 38A, 39A, 40A, or 41A may be extended by one or more amino acids at the N-terminus, C-terminus, or both, wherein at least one of the amino acids has a side chain that comprises a reactive functional group such as a primary or secondary amine, a sulfhydryl group, a carboxyl group (which may be present as a carboxylate group), a guanidino group, a phenol group, an indole ring, a thioether, or an imidazole ring, which facilitate conjugation with a reactive functional group to attach a CRM to the compstatin analog. It will be understood that a corresponding compstatin analog not comprising the CRM may also lack one or more such amino acids which are present in the long-acting compstatin analog to which it corresponds. Thus, a corresponding compstatin analog comprising any of SEQ ID NOs: 3-36, 37, 37A, 38A, 39A, 40A, or 41A and lacking a CRM will be understood to "have the same amino acid sequence" as SEQ ID NO: 3-36, 37, 37A, 38A, 39A, 40A, or 41A, respectively. For example, a corresponding compstatin analog comprising the amino acid sequence of SEQ ID NO: 14, 21, 28, 29, 32, 33, 34, or 36 and lacking a CRM will be understood to "have the same amino acid sequence" as SEQ ID NO: 14, 21, 28, 29, 32, 33, 34, or 36, respectively. A plasma half-life may be a terminal half-life after administration of a single IV dose. A plasma half-life may be a terminal half-life after steady state has been reached following administration of multiple IV doses. A long-acting compstatin analog may achieve a C_{max} in plasma at least 5-fold as great as that of a corresponding compstatin analog not comprising the CRM, e.g., between 5- and 50-fold as great, following administration of a single IV dose to a primate, or following administration of multiple IV doses. A long-acting compstatin analog may achieve a C_{max} in plasma between 10- and 20-fold as great as that of a corresponding compstatin analog not comprising the CRM following administration of a single IV dose to a primate, or following administration of multiple IV doses. In some embodiments a primate is human. In some embodiments a primate is a non-human primate, e.g., a monkey, such as a Cynomolgus monkey or Rhesus monkey. Renal clearance of a long-acting compstatin analog during the first 24 hours following administration IV at a dose of 10 mg/kg or 20 mg/kg to humans or to non-human primates may be reduced by at least a factor of 2, e.g., by a factor of 2-5, 5-10, 10-50, or 50-100 -fold or 100-150-fold or 150-200 fold as compared with renal clearance of a corresponding compstatin analog. Such a reduction in renal clearance may be observed following administration via other routes such as subcutaneous administration and/or using other doses, e.g., other doses described herein, e.g., 20 mg/kg. The concentration of compstatin analog can be measured in blood and/or urine samples using, e.g., UV, HPLC, mass spectrometry (MS) or antibody to the CRM, or combinations of such methods, such as LC/MS or LC/MS/MS. Pharmacokinetic parameters such as half-life and clearance can be determined using methods known to those of ordinary skill in the art. Pharmacokinetic analysis can be performed, e.g., with WinNonlin software v 5.2 (Pharsight Corporation, St. Louis, MO) or other suitable programs.

[0130] A CRM may be stable in physiological conditions for at least 24 hours or more. A CRM may be stable in mammalian, e.g., primate, e.g., human or non-human primate (e.g., monkey) blood, plasma, or serum for at least 24 hours. At least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more, of the CRM molecules may remain intact upon incubation in physiological conditions for 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, 168 hours, or more. At least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more, of the CRM molecules may remain intact upon incubation in blood, plasma, or serum at 37 degrees C for 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, 168 hours, or more. Incubation may be performed using a CRM at a concentration of between 1 microgram/ml

to about 100 mg/ml. Samples may be analyzed at various time points. Size or intactness may be assessed using, e.g., chromatography (e.g., HPLC), mass spectrometry, Western blot, or any other suitable method. Such stability characteristics may be conferred on a moiety conjugated to the CRM. A long-acting compstatin analog comprising a CRM may have any of the afore-mentioned stability characteristics. In some aspects intact with regard to a long-acting compstatin analog means that the compstatin analog moiety remains conjugated to the CRM and the CRM size remains about the same as at the start of incubation or administration.

[0131] A long-acting compstatin analog may have a molar activity of at least about 10%, 20%, 30%, e.g., between 30% and 40%, between 30% and 50%, between 30% and 60%, between 30% and 70%, between 30% and 80%, between 30% and 90%, or more, of the activity of a corresponding compstatin analog having the same amino acid sequence (and, if applicable, one or more blocking moiety(ies)) but not comprising a CRM. Wherein a long-acting compstatin analog comprises multiple compstatin analog moieties, the molar activity of the long-acting compstatin analog may be at least about 10%, 20%, or 30%, e.g., between 30% and 40%, between 30% and 50%, between 30% and 60%, between 30% and 70%, between 30% and 80%, between 30% and 90%, or more, of the sum of the activities of said compstatin analog moieties.

[0132] In the invention, the polyethylene glycol (PEG) comprises a $(\text{CH}_2\text{CH}_2\text{O})_n$ moiety and has a molecular weight of 40 kD. "Average molecular weight" refers to the number average molecular weight. The polydispersity D of a $(\text{CH}_2\text{CH}_2\text{O})_n$ moiety may be between 1.0005 and 1.50, e.g., between 1.005 and 1.10, 1.15, 1.20, 1.25, 1.30, 1.40, or 1.50, or any value between 1.0005 and 1.50.

[0133] In some embodiments, the $(\text{CH}_2\text{CH}_2\text{O})_n$ moiety is monodisperse and the polydispersity of a $(\text{CH}_2\text{CH}_2\text{O})_n$ moiety is 1.0. Such monodisperse $(\text{CH}_2\text{CH}_2\text{O})_n$ moieties are known in the art and are commercially available from Quanta BioDesign (Powell, OH).

[0134] In the invention, a compstatin analog moiety is attached at each end of the linear PEG. A bifunctional PEG having a reactive functional group at each end of the chain may be used, e.g., as described above. In some embodiments the reactive functional groups are identical while in some embodiments different reactive functional groups are present at each end. In general and compounds depicted herein, a polyethylene glycol moiety is drawn with the oxygen atom on the right side of the repeating unit or the left side of the repeating unit. In cases where only one orientation is drawn, the present invention encompasses both orientations (i.e., $(\text{CH}_2\text{CH}_2\text{O})_n$ and $(\text{OCH}_2\text{CH}_2)_n$) of polyethylene glycol moieties for a given compound or genus, or in cases where a compound or genus contains multiple polyethylene glycol moieties, all combinations of orientations are encompassed by the present disclosure.

[0135] Formulas of some disclosed monofunctional PEGs comprising a reactive functional group are illustrated below. For illustrative purposes, formulas in which the reactive functional group(s) comprise an NHS ester are depicted, but other reactive functional groups could be used, e.g., as described above. The $(\text{CH}_2\text{CH}_2\text{O})_n$ are depicted as terminating at the left end with a methoxy group (OCH_3) but it will be understood that the chains depicted below and elsewhere herein may terminate with a different OR moiety (e.g., an aliphatic group, an alkyl group, a lower alkyl group, or any other suitable PEG end group) or an OH group. It will also be appreciated that moieties other than those depicted may connect the $(\text{CH}_2\text{CH}_2\text{O})_n$ moieties with the NHS group.

[0136] A disclosed monofunctional PEG is of formula A:



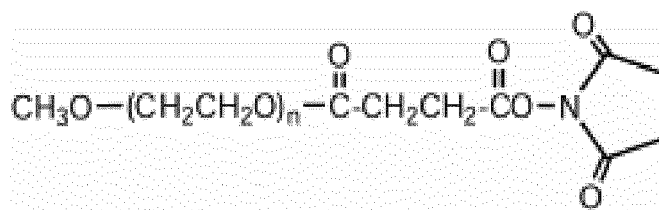
Formula A

wherein "Reactive functional group" and n are as defined above and described in classes and subclasses herein;

R^1 is hydrogen, aliphatic, or any suitable end group; and

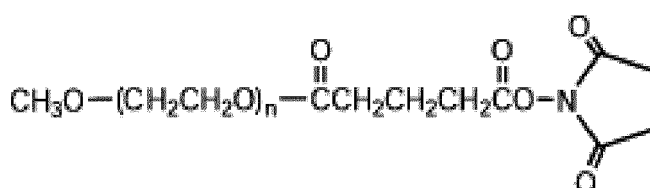
T is a covalent bond or a C_{1-12} straight or branched, hydrocarbon chain wherein one or more carbon units of T are optionally and independently replaced by $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}^x)-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})-$, $-\text{N}(\text{R}^x)\text{C}(\text{O})-$, $-\text{C}(\text{O})\text{N}(\text{R}^x)-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{N}(\text{R}^x)\text{SO}_2-$, or $-\text{SO}_2\text{N}(\text{R}^x)-$; and each R^x is independently hydrogen or C_{1-6} aliphatic.

[0137] Exemplary disclosed monofunctional PEGs of formula A include:

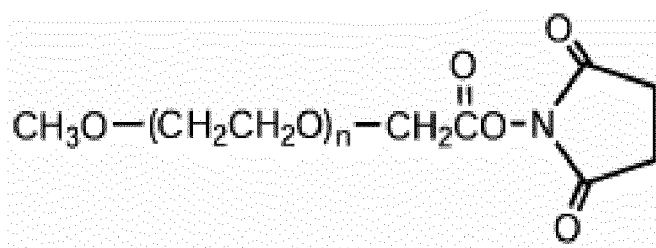


Formula I

[0138] In Formula I, the moiety comprising the reactive functional group has the general structure $-\text{CO}-(\text{CH}_2)_m-\text{COO}-\text{NHS}$, where $m=2$. A monofunctional PEGs may have the structure of Formula I, where m is between 1 and 10, e.g., between 1 and 5. For example, m may be 3, as shown below:

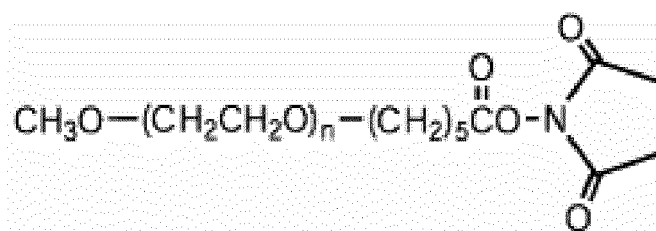


Formula Ia.

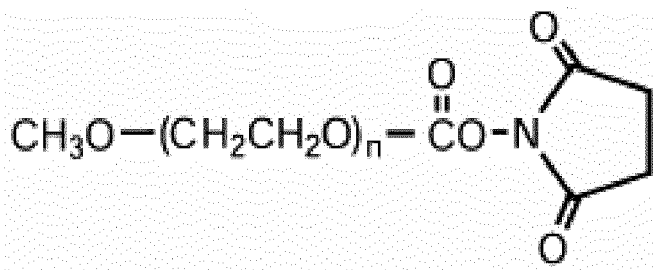


Formula II

[0139] In Formula II, the moiety comprising the reactive functional group has the general structure $-(\text{CH}_2)_m-\text{COO}-\text{NHS}$, where $m=1$. A monofunctional PEG may have the structure of Formula II, where m is between 1 and 10 (e.g., wherein m is 5 as shown in Formula III below), or wherein m is 0 (as shown below in Formula IIIa).



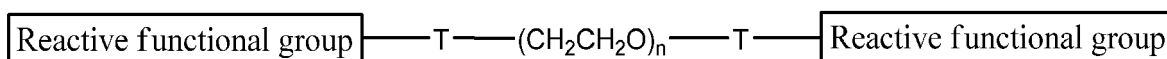
Formula III



Formula IIIa

[0140] A bifunctional linear PEG comprises a moiety comprising a reactive functional group at each of its ends. The reactive functional groups may be the same (homobifunctional) or different (heterobifunctional). The structure of a bifunctional PEG may be symmetric, wherein the same moiety is used to connect the reactive functional group to oxygen atoms at each end of the $-(\text{CH}_2\text{CH}_2\text{O})_n$ chain. Different moieties may be used to connect the two reactive functional groups to the PEG portion of the molecule. The structures of exemplary bifunctional PEGs are depicted below. For illustrative purposes, formulas in which the reactive functional group(s) comprise an NHS ester are depicted, but other reactive functional groups could be used.

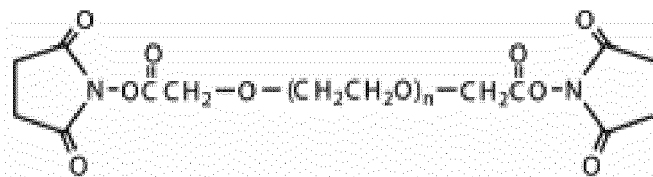
[0141] A bifunctional linear PEG may be of formula B:



Formula B

wherein each T and "Reactive functional group" is independently as defined above and described in classes and subclasses herein, and n is as defined above and described in classes and subclasses herein.

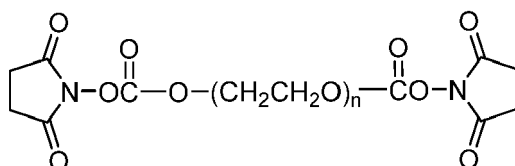
[0142] Exemplary bifunctional PEGs of formula B include:



Formula IV

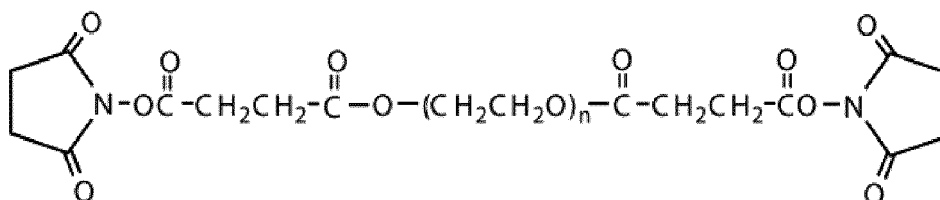
[0143] In Formula IV, the moiety comprising the reactive functional group has the general structure $-(\text{CH}_2)_m-\text{COO}-\text{NHS}$, where $m=1$. A bifunctional PEG may have the structure of Formula IV, where m is between 1 and 10, e.g., between 1 and 5. When m is 0, the moiety comprising the reactive functional group has the general structure $-\text{COO}-\text{NHS}$.

[0144] In one embodiment, the invention provides a compstatin analog obtainable by coupling two CA28-AEEAC-Lys moieties according to SEQ ID NO: 51 to a bifunctional linear PEG moiety having a molecular weight of 40 kDa, wherein the bifunctional PEG has the structure of Formula IVa, as shown below:



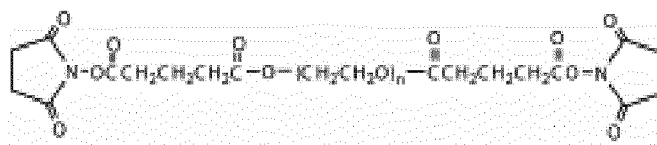
Formula IVa

[0145] Another disclosed bifunctional PEG is shown in Formula (V).



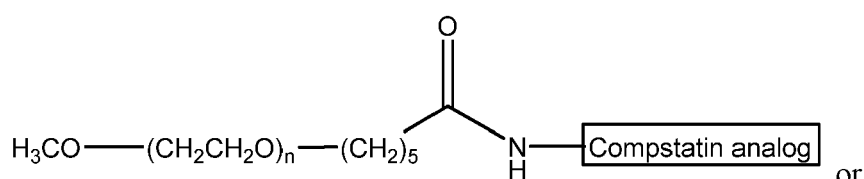
Formula V

[0146] In Formula V, the moiety comprising the reactive functional group has the general structure $-\text{CO}-(\text{CH}_2)_m-\text{COO}-\text{NHS}$, where $m=2$. Such bifunctional PEGs have the structure of Formula V, where m is between 1 and 10, e.g., between 1 and 5. For example, m may be 2, as shown below:

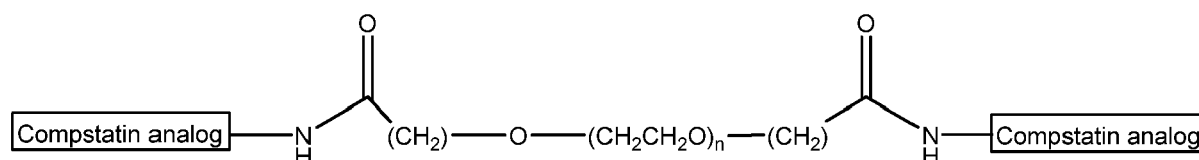


Formula Va

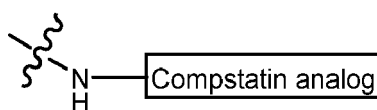
[0147] The present invention thus provides a compstatin analog conjugated to a polymer. A functional group (for example, an amine, hydroxyl, or thiol group) on a compstatin analog may be reacted with a PEG-containing compound having a "reactive functional group" as described herein, to generate such conjugates. By way of example, Formulae III and IV, respectively, can form compstatin analog conjugates having the structure:



or

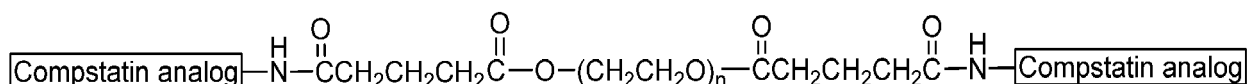
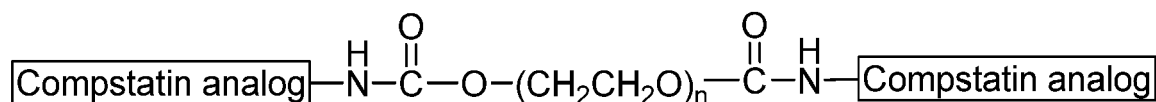


wherein,



represents the attachment point of an amine group on a compstatin analog. In certain embodiments, an amine group is a lysine side chain group.

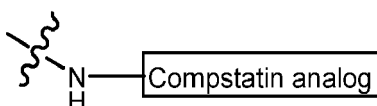
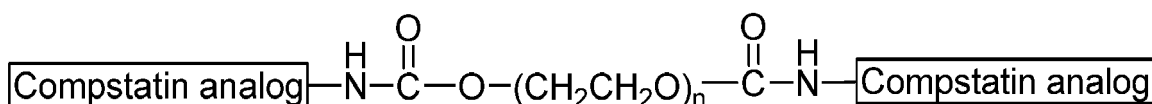
[0148] It will be appreciated that corresponding conjugates can be formed with any of the PEG-containing compounds and genera depicted herein, depending on the choice of reactive functional group and/or compstatin functional group. For example, Formulae IVa and Va, respectively, can form compstatin analog conjugates having the following structures



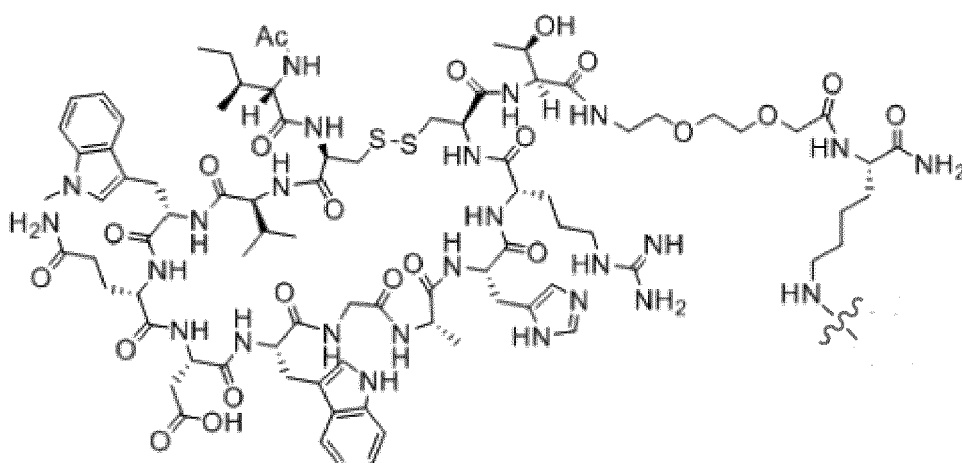
In the invention, the PEG component has an average molecular weight of 40 kD.

[0149] The term "bifunctional" or "bifunctionalized" is sometimes used herein to refer to a compound comprising two compstatin analog moieties linked to a CRM. Such compounds may be designated with the letter "BF". In the invention the bifunctionalized compound is symmetrical and the linkages between the CRM and each of the compstatin analog moieties are the same.

[0150] A linkage between a CRM and a compstatin analog of a bifunctionalized compound may comprise a carbamate. In some disclosed embodiments, each linkage between a CRM and a compstatin analog of a bifunctionalized compound may comprise a carbamate and not comprise an ester. Each compstatin analog of a bifunctionalized compound may be directly linked to a CRM via a carbamate. Each compstatin analog of a bifunctionalized compound may be directly linked to a CRM via a carbamate, and the bifunctionalized compound may have the structure:



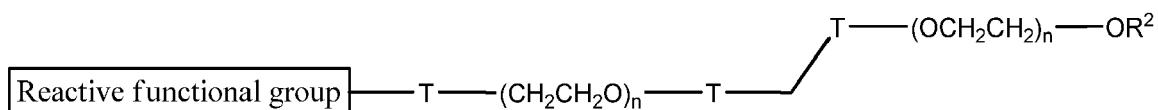
may represents point of attachment of a lysine side chain group in a compstatin analog having the structure:



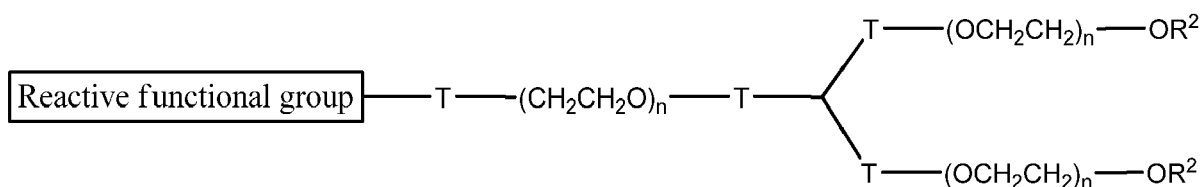
wherein the symbol "⋯" denotes the point of attachment of a chemical moiety to the remainder of a molecule or chemical formula.

[0151] Also disclosed is a branched, comb, or star-shaped PEG comprising a moiety comprising a reactive functional group at the end of each of multiple $-(\text{CH}_2\text{CH}_2\text{O})_n$ chains. The reactive functional groups may be the same or there may

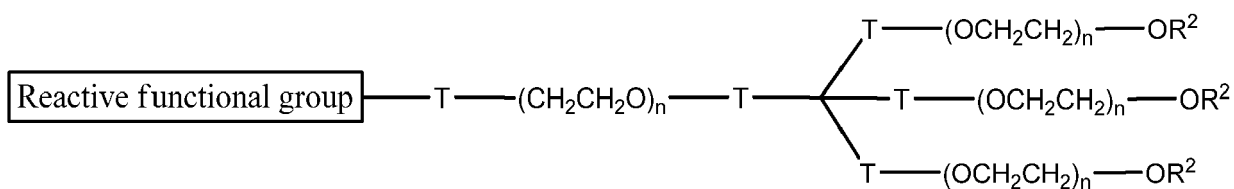
be at least two different groups. A disclosed branched, comb, or star-shaped PEG may be of the following formulae:



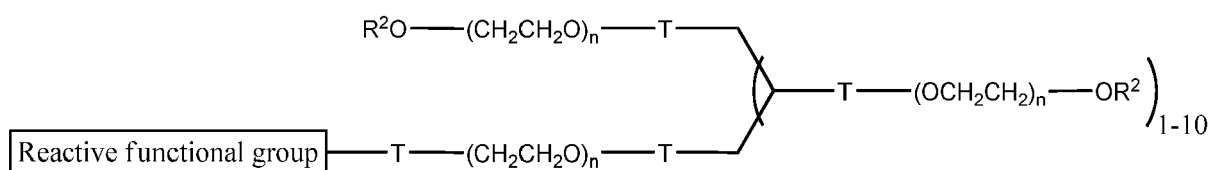
Formula C



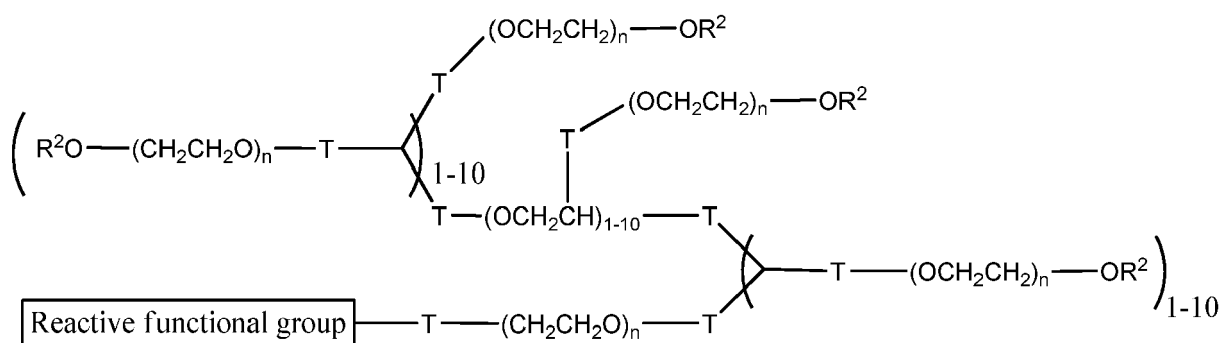
Formula D



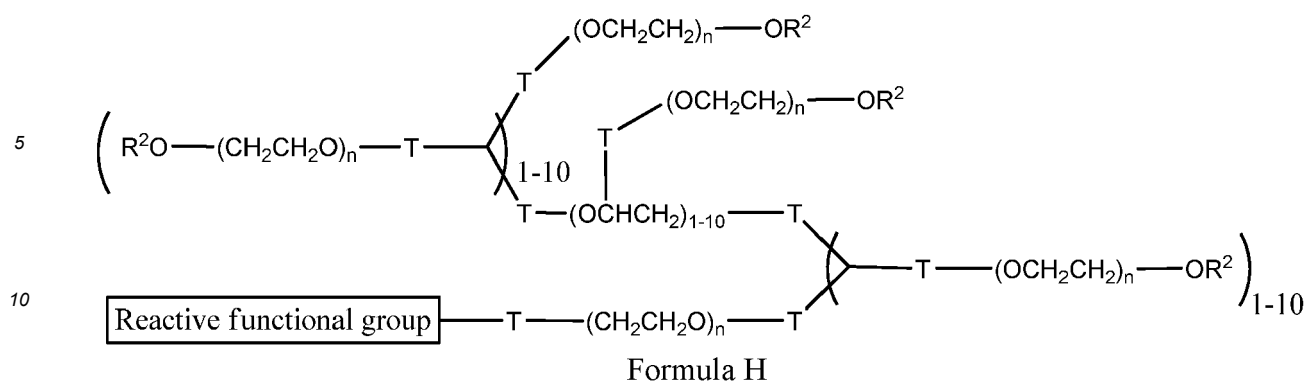
Formula E



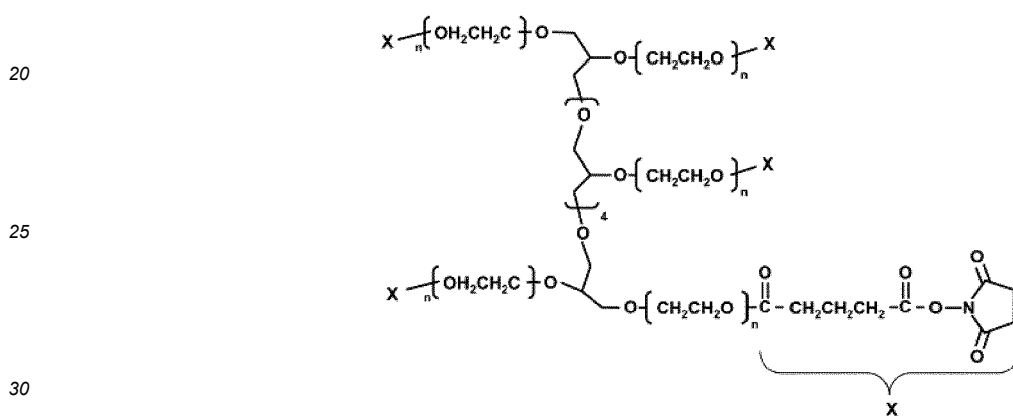
Formula F



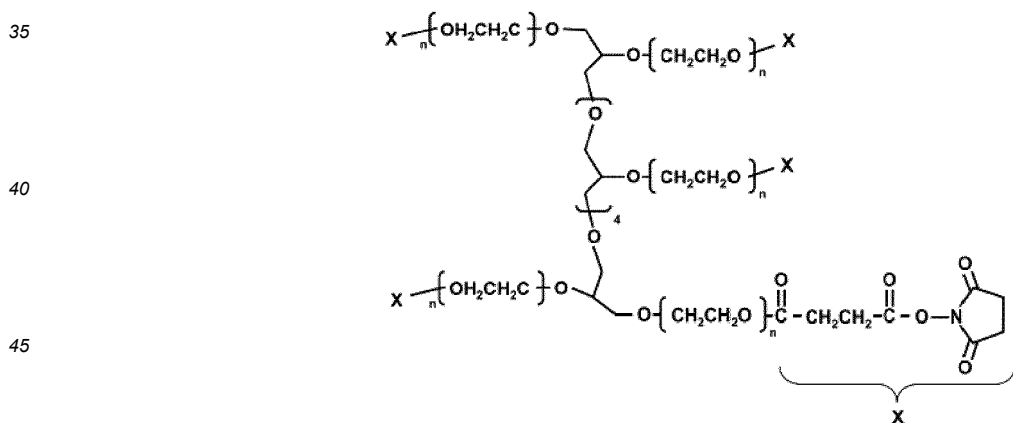
Formula G



[0152] wherein each R^2 is independently a "Reactive functional group" or R^1 , and each T, n, and "Reactive functional group" is independently as defined above and described in classes and subclasses herein. The structure of exemplary branched PEGs (having 8 arms, or branches) comprising NHS moieties as reactive functional groups is depicted below:

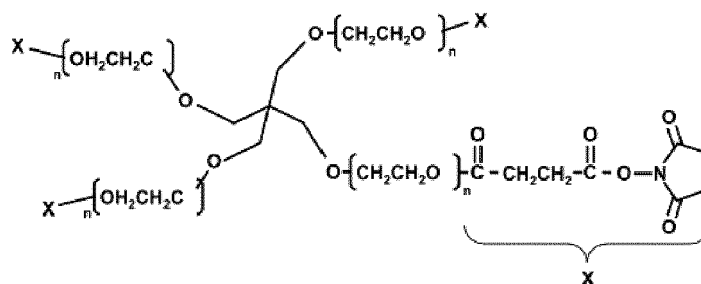


Formula VI

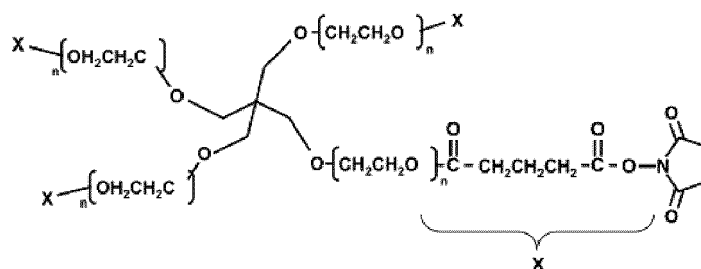


Formula VII

[0153] The structure of exemplary branched PEGs (having 4 arms, or branches) comprising NHS moieties as reactive functional groups is depicted below:



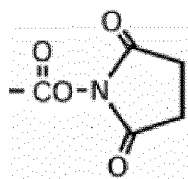
Formula VIII



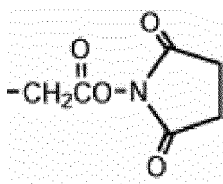
Formula IX

[0154] The number of branches emanating from the backbone may be varied. For example, the number 4 in the above formulae VI and VII may be changed to any other integer between 0 and 10.

[0155] A disclosed branched PEG has the structure of Formula VII, VIII, or IX (or variants thereof having different numbers of branches) with the proviso that x is



[0156] A disclosed branched PEG has the structure of Formula VII, VIII, or IX (or variants thereof having different numbers of branches) with the proviso that x is



[0157] Of course the methylene (CH₂) group in the above x moiety may instead comprise a longer alkyl chain (CH₂)_m, where m is up to 2, 3, 4, 5, 6, 8, 10, 20, or 30, or may comprise one or more other moieties described herein.

[0158] Further disclosed branched PEGs having NHS or maleimide reactive groups are depicted below:



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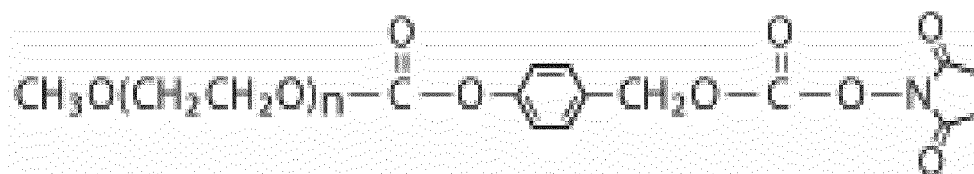
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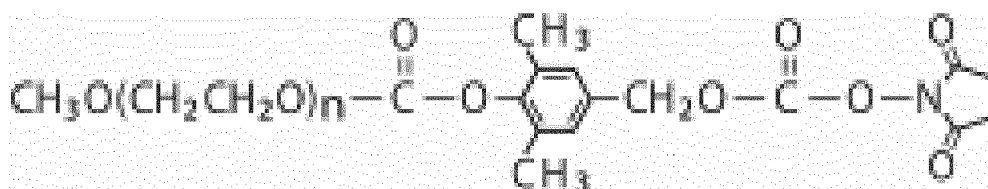
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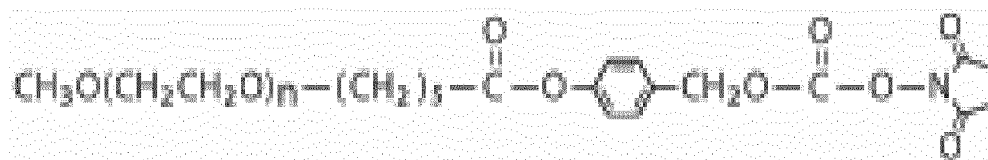
ester as a reactive functional group are as follows:



Formula XIV



Formula XV



Formula XVI

[0162] A disclosed branched (multi-arm) PEG or star-shaped PEG comprises a pentaerythritol core, hexaglycerin core, or tripentaerythritol core. It will be understood that the branches may not all emanate from a single point.

[0163] Monofunctional, bifunctional, branched, and other PEGs comprising one or more reactive functional groups may be obtained from, e.g., NOF America Corp. White Plains, NY or BOC Sciences 45-16 Ramsey Road Shirley, NY 11967, USA, among others, or may be prepared using methods known in the art.

[0164] A linkage between a CRM and a compstatin analog may comprise a carbamate. A compstatin analog may be directly linked to a CRM via a carbamate. In some disclosed embodiments, a linkage between a CRM and a compstatin analog does not comprise an ester. In some disclosed embodiments, a linkage between a CRM and a compstatin analog may comprise a carbamate and may not comprise an ester. A linkage between a CRM and a compstatin analog may comprise a carbamate and not comprise a bond that is more susceptible to hydrolysis in aqueous medium than a carbamate. The CRM may comprise or consist of a PEG moiety.

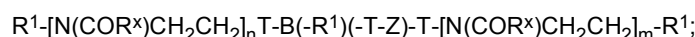
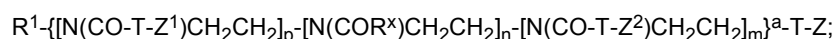
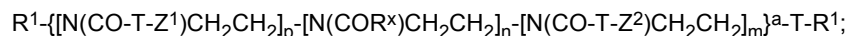
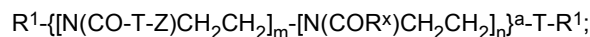
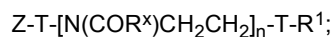
[0165] In the invention, each compstatin analog moiety is coupled to the CRM via an amide bond.

[0166] Also disclosed herein are one or more compstatin analogs of a multifunctionalized compound (e.g., a bifunctionalized, trifunctionalized, or more extensively functionalized compound) linked to a CRM by a linkage comprising a carbamate. In some embodiments, one or more compstatin analogs of such a multifunctionalized compound (e.g., a bifunctionalized, trifunctionalized, or more extensively functionalized compound) may be linked to a CRM by a linkage that does not comprise an ester. In some embodiments, one or more compstatin analogs of a multifunctionalized compound (e.g., a bifunctionalized, trifunctionalized, or more extensively functionalized compound) may be linked to a CRM by a linkage that comprises a carbamate and does not comprise an ester. One or more compstatin analogs of a multifunctionalized compound (e.g., a bifunctionalized, trifunctionalized, or more extensively functionalized compound) may be linked to a CRM by a linkage that comprises a carbamate and does not comprise a bond that is more susceptible to hydrolysis in aqueous medium than a carbamate. Each compstatin analog of a multifunctionalized compound (e.g., a bifunctionalized, trifunctionalized, or more extensively functionalized compound) may be directly linked to a CRM via a carbamate.

[0167] A CRM may comprise or consist of a PEG moiety. One or more compstatin analogs of a multifunctionalized compound (e.g., a bifunctionalized, trifunctionalized, or more extensively functionalized compound) may be linked to a CRM by a linkage comprising an amide. In some embodiments, one or more compstatin analogs of a multifunctionalized compound (e.g., a bifunctionalized, trifunctionalized, or more extensively functionalized compound) may be linked to a CRM by a linkage that comprises an amide and does not comprise an ester. One or more compstatin analogs of a multifunctionalized compound (e.g., a bifunctionalized, trifunctionalized, or more extensively functionalized compound)

may be linked to a CRM by a linkage that comprises an amide and does not comprise a bond that is more susceptible to hydrolysis in aqueous medium than an amide. Each compstatin analog of a multifunctionalized compound (e.g., a bifunctionalized, trifunctionalized, or more extensively functionalized compound) may be directly linked to a CRM via an amide. The CRM may comprise or consist of a PEG moiety.

[0168] Also disclosed is a compstatin analog conjugated with a polymer, wherein the polymer is other than PEG. Such a polymer may be a polyoxazoline (POZ). Exemplary mono- and poly-functionalized polyoxazoline derivatives for direct conjugation, or for conjugation via a linker, are depicted below:



wherein:

each of Z, Z¹ and Z² is independently a reactive functional group as defined above and described in classes and subclasses herein;

each of T, R^x, and R¹ is independently as defined above and described in classes and subclasses herein;

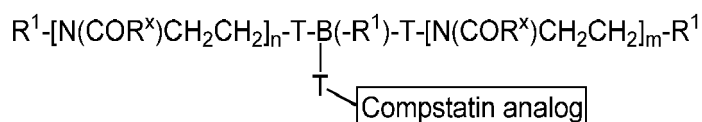
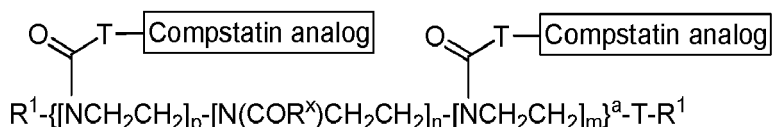
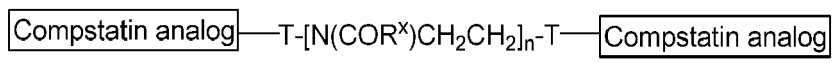
each of m, n, and p is independently an integer 0-1000, with the limitation that the sum of m, n, and p for each formula is not 0;

a is "ran," which indicates a random copolymer, or "block," which indicates a block copolymer;

B is a branching moiety that is linked with or without a linker to the other parts of the polymer.

[0169] Other examples of functionalized polyoxazoline derivatives for conjugation are extensively described in the art, including but not limited to those described in PCT Patent Application Publication Nos. WO/2010/006282, WO/2009/089542, WO/2009/043027 and WO/2008/106186.

[0170] Exemplary compstatin analog conjugates with polyoxazoline polymers are depicted below:



wherein each variable is independently as defined above and described in classes and subclasses herein.

[0171] Further disclosed is a compstatin analog conjugated with a polymer, wherein the compstatin analog is connected to the polymer via one or more linkers. A polymer may be selected from PEG-containing compounds and genera described above and in classes and subclasses herein. Compstatin analog conjugates of PEG-containing compounds and genera depicted herein, wherein the compstatin analog is connected to the PEG-containing moieties via one or more linkers, are further disclosed. Mono- and poly-functional PEGs that comprise one or more reactive functional groups for conju-

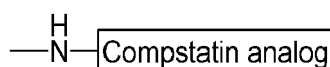
gation are defined above and described in classes and subclasses herein, including but not limited to those of formula A, I, Ia, II, III, IIIa, B, IV, IVa, V, Va, C, D, E, F, G, H, VI, VII, VIII, IX, X, XI, XII, XIII, XIV, XV, or XVI.

[0172] Disclosed linkers for connecting a compstatin analog and a polymer moiety such as PEG or polyoxazoline are extensively described above and in classes and subclasses herein. A linker may have multiple functional groups, wherein one functional group is connected to a compstatin analog and another is connected to a polymer moiety. A linker may be a bifunctional compound. A linker may have the structure of $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{C}(=\text{O})\text{OH}$, wherein n is 1 to 1000. A disclosed linker comprises 8-amino-3,6-dioxaoctanoic acid (AEEAc). A disclosed linker may be activated for conjugation with a polymer moiety or a functional group of a compstatin analog. For example, the carboxyl group of AEEAc may be activated before conjugation with the amine group of the side chain of a lysine group.

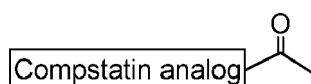
[0173] A suitable functional group (for example, an amine, hydroxyl, thiol, or carboxylic acid group) on a compstatin analog may be used for conjugation with a polymer moiety, either directly or via a linker. A compstatin analog may be conjugated through an amine group to a PEG moiety via a linker. An amine group may be the α -amino group of an amino acid residue. An amine group may be the amine group of the lysine side chain. A compstatin analog may be conjugated to a PEG moiety through the amino group of a lysine side chain (ϵ -amino group) via a linker having the structure of $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{C}(=\text{O})\text{OH}$, wherein n is 1 to 1000. A compstatin analog may be conjugated to a PEG moiety through the amino group of a lysine side chain via a linker comprising AEEAc. A $\text{NH}_2(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{C}(=\text{O})\text{OH}$ linker may introduce a $-\text{NH}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{C}(=\text{O})-$ moiety on a compstatin lysine side chain after conjugation. A linker comprising AEEAc may introduce a $-\text{NH}(\text{CH}_2\text{CH}_2\text{O})_2\text{CH}_2\text{C}(=\text{O})-$ moiety on a compstatin lysine side chain after conjugation.

[0174] In the invention, each compstatin analog moiety is conjugated to the polymer moiety via a linker comprising an AEEAc moiety and a lysine residue. The polymer is PEG. The C-terminus of each compstatin analog is connected to the amino group of AEEAc, and the C-terminus of AEEAc is connected to a lysine residue. The C-terminus of each compstatin analog is connected to the amino group of AEEAc, and the C-terminus of AEEAc is connected to the α -amino group of a lysine residue. The C-terminus of a compstatin analog is connected to the amino group of AEEAc, the C-terminus of AEEAc is connected to the α -amino group of the lysine residue, and the PEG moiety is conjugated through the ϵ -amino group of said lysine residue. The C-terminus of the lysine residue may be modified e.g. by amidation. The N-terminus of each compstatin analog is acetylated.

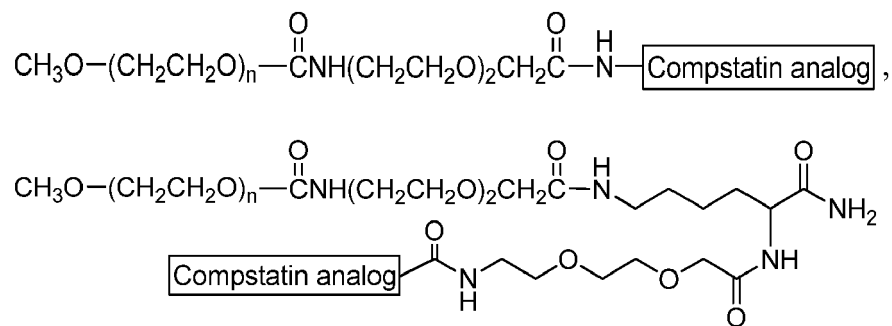
[0175] Further conjugates comprising an AEEAc linker and a polymer are depicted below, wherein



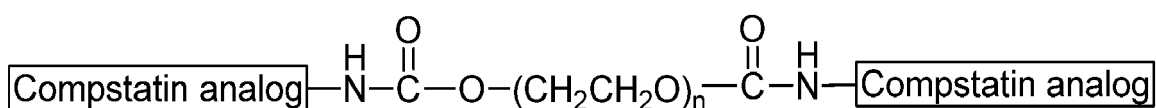
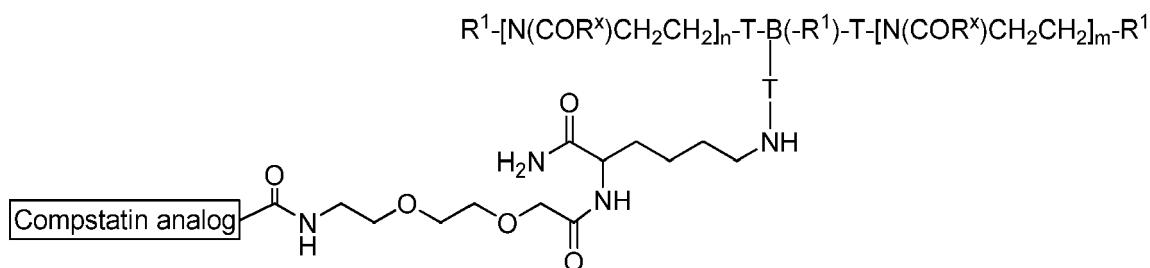
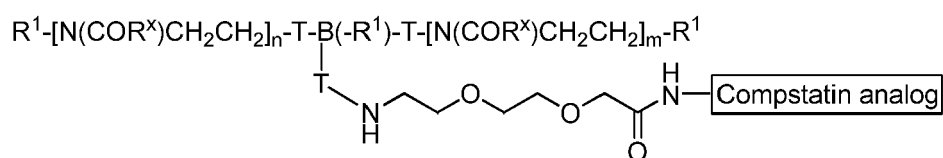
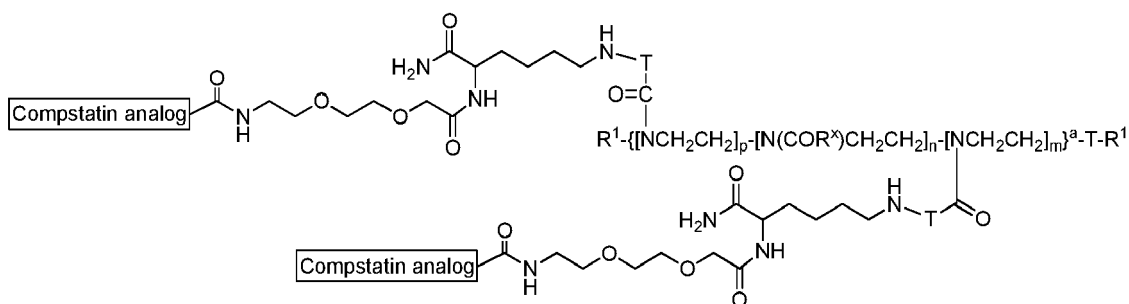
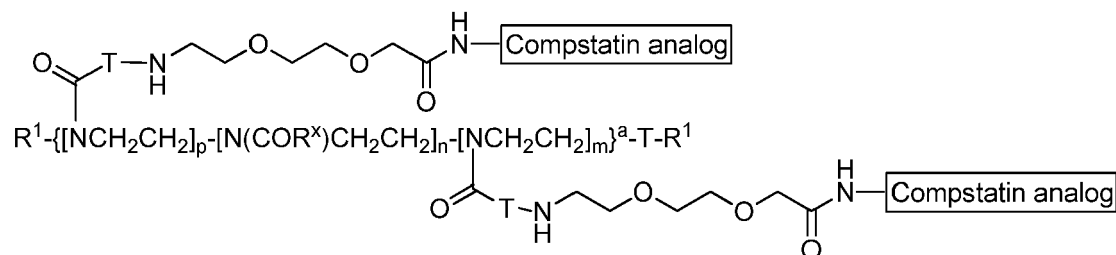
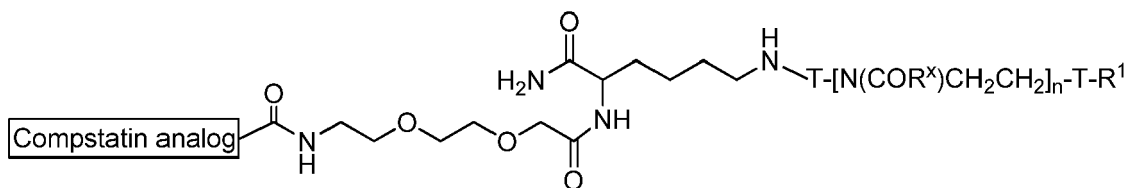
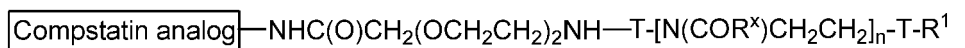
represents the attachment point of an amine group on a compstatin analog,

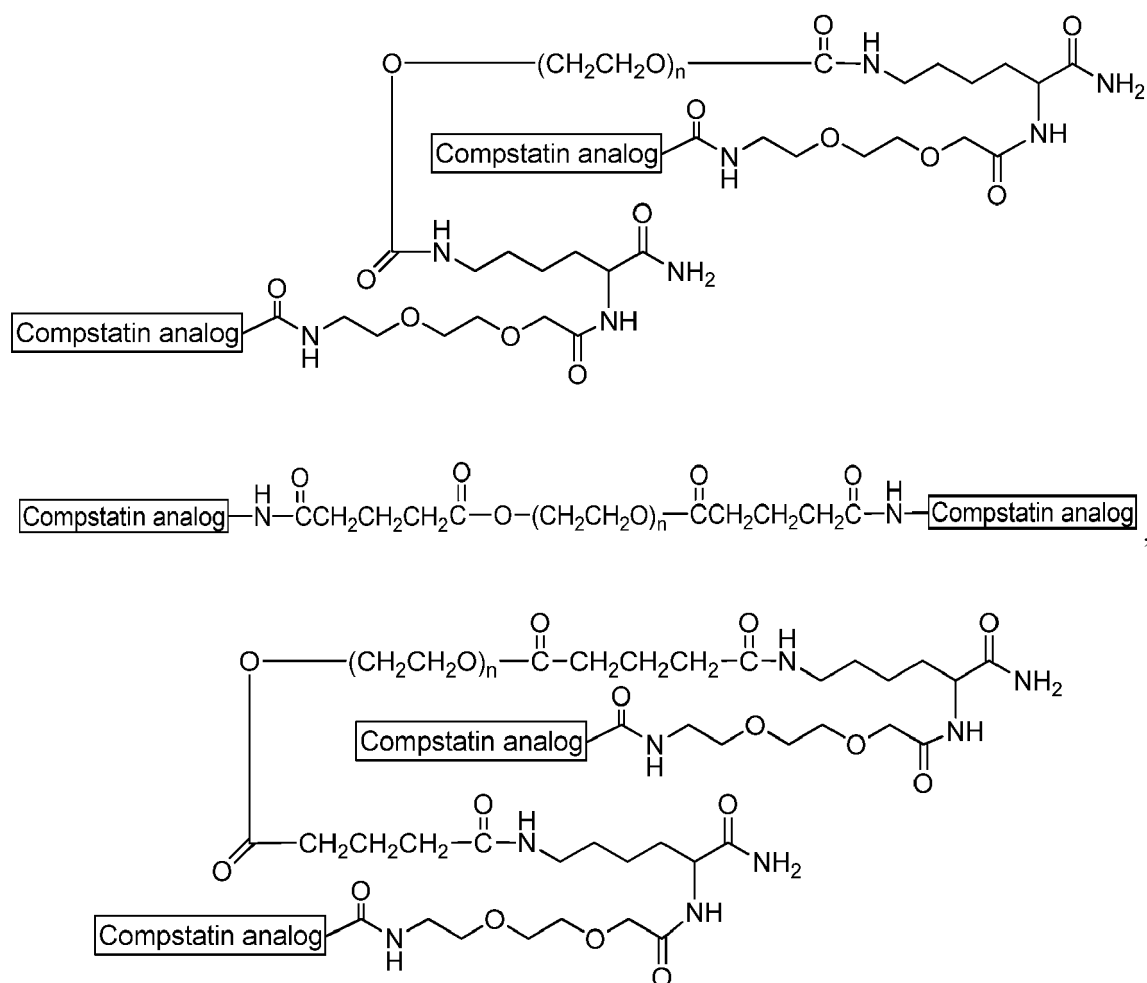


represents a compstatin analog attaching through its C-terminus, and wherein each of the other variables is independently as defined above and described in classes and subclasses herewith. In some embodiments, an amine group is the amino group of a lysine side chain.









[0176] A compstatin analog may be represented as M-AEEAc-Lys- B_2 , wherein B_2 is a blocking moiety, e.g., NH_2 , M represents any of SEQ ID NOs: 3-36, 37, 37A, 38A, 39A, 40A, or 41A, with the proviso that the C-terminal amino acid of any of SEQ ID NOs: 3-36, 37, 37A, 38A, 39A, 40A, or 41A is linked via a peptide bond to AEEAc-Lys- B_2 . The NHS moiety of a monofunctional or multifunctional (e.g., bifunctional) PEG may react with the free amine of the lysine side chain to generate a monofunctionalized (one compstatin analog moiety) or multifunctionalized (multiple compstatin analog moieties) long-acting compstatin analog. Any amino acid comprising a side chain that comprises a reactive functional group may be used instead of Lys (or in addition to Lys). A monofunctional or multifunctional PEG comprising a suitable reactive functional group may be reacted with such side chain in a manner analogous to the reaction of NHS-ester activated PEGs with Lys.

[0177] With regard to any of the above formulae and structures, it is to be understood that embodiments in which the compstatin analog component comprises any compstatin analog described herein, e.g., any compstatin analog of SEQ ID NOs: 3-36, 37, 37A, 38A, 39A, 40A, 41A, are expressly disclosed. For example, and without limitation, a compstatin analog may comprise the amino acid sequence of SEQ ID NO: 28. An exemplary long-acting compstatin analog in which the compstatin analog component comprises the amino acid sequence of SEQ ID NO: 28 is depicted in Figure 10(C). It will be understood that the PEG moiety may have a variety of different molecular weights or average molecular weights in various embodiments, as described herein. For example, individual PEG chains within a preparation may vary in molecular weight and/or different preparations may have different average molecular weights and/or polydispersity, as described herein. In certain embodiments, the PEG moiety in the compound of Figure 10(C) has an average molecular weight of between about 20 kD -100 kD, about 20 kD-90 kD, about 20 kD-80 kD, about 20 kD-70 kD, about 20 kD-60 kD, about 20 kD-50 kD, about 30 kD-80 kD, about 30 kD-70 kD, about 30 kD-60 kD, about 30 kD-50 kD, about 30 kD-45 kD, about 35 kD-50 kD, about 35 kD-45 kD, about 36 kD-44 kD, about 37 kD-43 kD, about 38 kD-42 kD, or about 39 kD-41 kD. In some embodiments the PEG moiety in the compound of Figure 10(C) has an average molecular weight between about 30 kD and about 50 kD, e.g., between about 35 kD and about 45 kD, between about 37.5 kD and about 42.5 kD. In certain embodiments in which the PEG moiety has an average molecular weight of about 40 kD, e.g., 37.5 kD - 42.5 kD, 38 kD, 39 kD, 40 kD, 41 kD, 42 kD, the compound is sometimes referred to herein as CA28-2TS- BF. In

certain embodiments a compound comprising a CRM, e.g., a PEG moiety, that has an average molecular weight of about 40 kD, e.g., 37.5 kD - 42.5 kD, 38 kD, 39 kD, 40 kD, 41 kD, 42 kD, the compound has a terminal half-life of at least about 5 days, e.g., about 5-10 days, e.g., about 5, 6, 7, 8, 9 days, when administered IV or subcutaneously to non-human primates or humans, e.g., at a dose of about 1-3 mg/kg, 3-5 mg/kg, or 5-10 mg/kg.

[0178] Disclosed compstatin analogs may comprise both a cell-reactive functional group and a CRM. A cell-reactive functional group or moiety may be replaced by a $(\text{CH}_2\text{CH}_2\text{O})_n$ moiety having a molecular weight of at least 500 daltons, e.g., at least 1,500 daltons up to about 100,000 daltons (e.g., an average molecular weight of about 20,000; 30,000; 40,000; 50,000; 60,000; 70,000; 80,000; 90,000; or 100,000 daltons). The average molecular weight of the compound or $(\text{CH}_2\text{CH}_2\text{O})_n$ moieties may be at least 20,000 daltons, up to about 100,000; 120,000; 140,000; 160,000; 180,000; or 200,000 daltons.

[0179] Further disclosed long-acting compstatin analogs are set forth below, wherein n is sufficient to provide an average molecular weight of between about 500; 1,000; 1,500; 2,000; 5,000; 10,000; 20,000; 30,000; 40,000; 50,000; 60,000; 70,000; 80,000; 90,000; and 100,000 daltons. n may be sufficient to provide an average molecular weight of between about 20,000 daltons, up to about 100,000; 120,000; 140,000; 160,000; 180,000; or 200,000 daltons.

$(\text{CH}_2\text{CH}_2\text{O})_n\text{C}(=\text{O})\text{-Ile-Cys-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys-Thr- NH}_2$ (SEQ ID NO: 58)

Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-NH- $\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{-C}(=\text{O})\text{-Lys-C}(=\text{O})\text{-(CH}_2\text{CH}_2\text{O})_n\text{-NH}_2$ (SEQ ID NO: 59)

Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-Lys-C($=\text{O}$)- $(\text{CH}_2\text{CH}_2\text{O})_n\text{-NH}_2$ (SEQ ID NO: 60).

Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-(Gly)₅-Lys-C($=\text{O}$)- $(\text{CH}_2\text{CH}_2\text{O})_n\text{-NH}_2$ (SEQ ID NO: 61)

Ac- $(\text{CH}_2\text{CH}_2\text{O})_n\text{C}(=\text{O})\text{-Lys-(Gly)}_5\text{-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-NH}_2$ (SEQ ID NO: 62)

Ac- $(\text{CH}_2\text{CH}_2\text{O})_n\text{C}(=\text{O})\text{-Lys-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr- NH}_2$ (SEQ ID NO: 63)

[0180] In SEQ ID NO: 58, the $(\text{CH}_2\text{CH}_2\text{O})_n$ is coupled via an amide bond to the N-terminal amino acid. In SEQ ID NOs: 59-63, the $(\text{CH}_2\text{CH}_2\text{O})_n$ moiety is coupled via an amide bond to a Lys side chain; thus it will be understood that the NH_2 at the C-terminus in SEQ ID NOs: 59, 60, and 61, represents amidation of the C-terminus of the peptide, and it will be understood that in SEQ ID NOs: 62 and 63, the Ac at the N-terminus represents acetylation of the N-terminus of the peptide, as described above. It will also be appreciated by those of ordinary skill in the art that a free end of a $(\text{CH}_2\text{CH}_2\text{O})_n$ moiety typically terminates with an OR where the underlined O represents the O atom in the terminal $(\text{CH}_2\text{CH}_2\text{O})$ group. (OR) is often a moiety such as a hydroxyl (OH) or methoxy ($-\text{OCH}_3$) group though other groups (e.g., other alkoxy groups) could be used. Thus SEQ ID NO: 59, for example, may be represented as Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-NH- $\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{-C}(=\text{O})\text{-Lys-C}(=\text{O})\text{-(CH}_2\text{CH}_2\text{O})_n\text{-R)-NH}_2$ (SEQ ID NO: 64) wherein R is, e.g., either H or CH_3 in the case of a linear PEG. In the case of a bifunctional, branched or star-shaped PEG, R represents the remainder of the molecule. Further, it will be understood that the moiety comprising the reactive functional group may vary, as described herein (e.g., according to any of the formulas described herein). For example, long-acting compstatin analogs comprising the same peptide sequence as SEQ ID NO: 64, in which the moiety comprising the reactive functional group comprises an ester and/or alkyl chain may be represented as follows

Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-NH- $\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{-C}(=\text{O})\text{-Lys-C}(=\text{O})\text{-(CH}_2\text{)}_m\text{-(CH}_2\text{CH}_2\text{O})_n\text{-R)-NH}_2$ (SEQ ID NO: 65);

Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-NH- $\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{-C}(=\text{O})\text{-Lys-C}(=\text{O})\text{-(CH}_2\text{)}_m\text{-C}(=\text{O})\text{-(CH}_2\text{CH}_2\text{O})_n\text{-R)-NH}_2$ (SEQ ID NO: 66)

Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-NH- $\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{-C}(=\text{O})\text{-Lys-C}(=\text{O})\text{-(CH}_2\text{)}_m\text{-C}(=\text{O})\text{-(CH}_2\text{)}_j\text{-(CH}_2\text{CH}_2\text{O})_n\text{-R)-NH}_2$ (SEQ ID NO: 67)

[0181] In SEQ ID NOs: 65-67 m may range from 1 up to about 2, 3, 4, 5, 6, 7, 8, 10, 15, 20, or 30 in various embodiments. In SEQ ID NOs: 67 j may range from 1 up to about 2, 3, 4, 5, 6, 7, 8, 10, 15, 20, or 30.

[0182] It will also be appreciated that, as described herein, other moieties may be incorporated between the Lys-C($=\text{O}$)- and $(\text{CH}_2\text{CH}_2\text{O})_n\text{-R}$, such as an amide, aromatic ring (e.g., a substituted or unsubstituted phenyl), or a substituted or unsubstituted cycloalkyl structure.

[0183] Disclosed herein are variants of SEQ ID NOs: 58-67 in which -Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr- is replaced by an amino acid sequence comprising the amino acid sequence of any other compstatin analog, e.g., of any of SEQ ID NOs 3-27 or 29-36, 37, 37A, 38A, 39A, 40A, or 41A with the proviso that blocking moiety(ies) present at the N- and/or C-termini of a compstatin analog may be absent, replaced by a linker (which may comprise a blocking moiety), or attached to a different N- or C-terminal amino acid present in the corresponding variant(s).

[0184] Any compstatin analog, e.g., any compound comprising any of SEQ ID NOs: 3-37, 37A, 38A, 39A, 40A, or 41A

maybe attached via or near its N-terminal or C-terminal end (e.g., via a side chain of an amino acid at or near its N-terminal or C-terminal amino acid) directly or indirectly to any moiety comprising a reactive functional group, e.g., any compound of Formulae I - XVI or Formulae A-H.

[0185] Other disclosed CRMs comprise a polypeptide that occurs in human serum, or a fragment thereof or a substantially similar variant of the polypeptide or fragment thereof. Such a polypeptide, fragment, or variant may have a molecular weight of between 5 kD and 150 kD, e.g., at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 kD, or more, e.g., between 100 and 120, or 120 and 150 kD. Producing a long-acting compstatin analog may comprise reacting a compstatin analog comprising a reactive functional group with one or more amino acid side chains of the polypeptide, wherein the side chain comprises a compatible functional group. Producing a long-acting compstatin analog may comprise reacting a compstatin analog comprising a reactive functional group with the N-terminal amine and/or C-terminal carboxyl group of the polypeptide. Producing a long-acting compstatin analog may comprise reacting a compstatin analog comprising an amine-reactive functional group with amino acids having a side chain comprising a primary amine (e.g., lysine) and/or with the N-terminal amine of the polypeptide. Producing a long-acting compstatin analog may comprise reacting a compstatin analog comprising a carboxyl-reactive functional group with the C-terminal carboxyl group of the polypeptide. A compstatin analog moiety may be attached at each terminus of the polypeptide and, optionally, to the side chain of one or more internal amino acids. Producing a long-acting compstatin analog may thus comprise reacting a compstatin analog comprising a sulfhydryl-reactive functional group with one or more sulfhydryl groups of the polypeptide.

[0186] In some disclosed aspects, at least one reactive functional group is introduced into the polypeptide. For example, in some aspects of the disclosure at least one side chain of the polypeptide is modified to convert a first reactive functional group to a different reactive functional group prior to reaction with the compstatin analog. In some aspects of the disclosure a thiol is introduced. Several methods are available for introducing thiols into biomolecules, including the reduction of intrinsic disulfides, as well as the conversion of amine, aldehyde or carboxylic acid groups to thiol groups. Disulfide crosslinks of cystines in proteins can be reduced to cysteine residues by dithiothreitol (DTT), tris-(2-carboxyethyl)phosphine (TCEP), or tris-(2-cyanoethyl)phosphine. Amines can be indirectly thiolated by reaction with succinimidyl 3-(2-pyridyldithio)propionate (SPDP) followed by reduction of the 3-(2-pyridyldithio)propionyl conjugate with DTT or TCEP. Amines can be indirectly thiolated by reaction with succinimidyl acetylthioacetate followed by removal of the acetyl group with 50 mM hydroxylamine or hydrazine at near-neutral pH. Amines can be directly thiolated by reaction with 2-iminothiolane, which preserve the overall charge of the molecule and introduces a free thiol. Tryptophan residues in thiol-free proteins can be oxidized to mercaptotryptophan residues, which can then be modified by iodoacetamides or maleimides. A polypeptide comprising one or more thiols may be reacted with a compstatin analog comprising a maleimide group, such as Ac-Ile-Cys*-Val-Trp(1-Me)-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-AEEAc-Lys-(C(=O)-(CH₂)₅-Mal)-NH₂ (SEQ ID NO: 68) to generate a long-acting compstatin analog.

[0187] In some aspects of the disclosure the polypeptide is recombinantly produced. In some aspects of the disclosure the polypeptide is at least in part recombinantly produced (e.g., in bacteria or in eukaryotic host cells such as fungal, insect, plant, or vertebrate) and/or at least in part produced using chemical synthesis. The polypeptide may be purified. For example, in some aspects of the disclosure the polypeptide is purified from a host cell lysate or from culture medium into which it has been secreted by host cells. In some aspects of the disclosure the polypeptide is glycosylated. In some aspects of the disclosure the polypeptide is non-glycosylated. In some aspects of the disclosure the polypeptide is human serum albumin (HSA). In some aspects of the disclosure a substantially similar variant of the polypeptide is sufficiently similar to the polypeptide of which it is a variant so as to not be recognized as foreign by a normal immune system of a subject, e.g., a human subject. In some aspects of the disclosure alterations in the sequence of substantially similar variant as compared with the polypeptide of which it is a variant are selected so as to avoid generating MHC Class I epitopes. Various methods known in the art can be used to predict whether a sequence comprises an MHC Class I epitope.

[0188] In some aspects of the disclosure, one or more amino acids in a polypeptide or linker or composition may be selected to be hydrophobic or hydrophilic or selected to confer increased hydrophilicity or increased hydrophobicity, on a compound that contains it. As known in the art, the terms "hydrophilic" and "hydrophobic" are used to refer to the degree of affinity that a substance has with water. In some aspects a hydrophilic substance has a strong affinity for water, tending to dissolve in, mix with, or be wetted by water, while a hydrophobic substance substantially lacks affinity for water, tending to repel and not absorb water and tending not to dissolve in or mix with or be wetted by water. Amino acids can be classified based on their hydrophobicity as well known in the art. Examples of "hydrophilic amino acids" are arginine, lysine, threonine, alanine, asparagine, glutamine, aspartate, glutamate, serine, and glycine. Examples of "hydrophobic amino acids" are tryptophan, tyrosine, phenylalanine, methionine, leucine, isoleucine, and valine. An analog of a standard amino acid may be used, wherein the analog has increased or decreased hydrophilic or hydrophobic character as compared with the amino acid of which it is an analog.

[0189] The compstatin analog of the invention comprises a linear PEG moiety attached to two compstatin analog moieties. Also disclosed are multimers, e.g., concatamers, comprising two or more (e.g., between 2 and 10) compstatin analogs comprising a CRM, wherein the average molecular weight of the resulting molecule (or the CRM components thereof) is between 20,000; 30,000; 40,000; 50,000; 60,000; 70,000; 80,000; 90,000; and 100,000 daltons. The average

molecular weight of the resulting molecule (or the CRM components thereof) may be at least 20,000 daltons, up to about 100,000; 120,000; 140,000; 160,000; 180,000; or 200,000 daltons. The compstatin analogs comprising a CRM can be linked using any of the linking moieties described above.

[0190] A wide variety of methods and assays useful for detection of polymers, e.g., PEGs, POZs, and/or polypeptides and/or useful for measurement of physical and/or structural properties of polymers, e.g., PEGs, POZs, and/or polypeptides are known in the art and may, if desired, be used to detect a compstatin analog, e.g., a cell-reactive, long-acting, targeted compstatin analog or a compstatin analog moiety. For example, methods and assays useful for determining properties such as aggregation, solubility, size, structure, melting properties, purity, presence of degradation products or contaminants, water content, hydrodynamic radius, etc., are available. Such methods include, e.g., analytical centrifugation, various types of chromatography such as liquid chromatography (e.g., HPLC-ion exchange, HPLC-size exclusion, HPLC-reverse phase), light scattering, capillary electrophoresis, circular dichroism, isothermal calorimetry, differential scanning calorimetry, fluorescence, infrared (IR), nuclear magnetic resonance (NMR), Raman spectroscopy, refractometry, UV/Visible spectroscopy, mass spectrometry, immunological methods, etc. It will be understood that methods may be combined. A cell-reactive, long-acting, or targeted compstatin analog (or composition comprising a cell-reactive, long-acting, or targeted compstatin analog) may have one or more properties described herein, as assessed using any of the foregoing methods. Methods useful to detect and/or quantify a long-acting compstatin analog are described herein.

VII. Targeted Compstatin Analogs

[0191] Also disclosed are targeted compstatin analogs that comprise a targeting moiety and a compstatin analog moiety, wherein the targeting moiety binds non-covalently to a target molecule. Targeted compstatin analogs may be analogous to the cell-reactive compstatin analogs described in Section VI, wherein the compounds comprise a targeting moiety in addition to, or instead of, a cell-reactive moiety. The targeting moiety can comprise, e.g., an antibody, polypeptide, peptide, nucleic acid (e.g., an aptamer), carbohydrate, small molecule, or supramolecular complex, that specifically binds to the target molecule. The affinity (as measured by the equilibrium dissociation constant, K_d) of targeting moiety for the target molecule (as measured by the equilibrium dissociation constant, K_d) may be 10^{-3} M or less, e.g., 10^{-4} M or less, e.g., 10^{-5} M or less, e.g., 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, or 10^{-9} M or less under the conditions tested, e.g., under physiological conditions.

[0192] In disclosed aspects in which the targeting moiety is an antibody, the antibody may be any immunoglobulin or a derivative thereof, which maintains binding ability, or any protein having a binding domain which is homologous or largely homologous to an immunoglobulin binding domain. Such proteins may be derived from natural sources, or partly or wholly synthetically produced (e.g., using recombinant DNA techniques, chemical synthesis, etc.). The antibody can be of any species, e.g., human, rodent, rabbit, goat, chicken, etc. The antibody may be a member of any immunoglobulin class, including any of the human classes: IgG, IgM, IgA, IgD, and IgE. The antibody may be a fragment of an antibody such as an Fab', F(ab')₂, scFv (single-chain variable) or other fragment that retains an antigen binding site, or a recombinantly produced scFv fragment, including recombinantly produced fragments. See, e.g., Allen, T., *Nature Reviews Cancer*, Vol. 2, 750-765, 2002, and references therein. Monovalent, bivalent or multivalent antibodies can be used. The antibody may be a chimeric antibody in which, for example, a variable domain of rodent origin is fused to a constant domain of human origin, thus retaining the specificity of the rodent antibody. A human antibody or portion thereof may be generated, for example, in rodents whose genome incorporates human immunoglobulin genes, using a display technology such as phage display, etc. A humanized antibody may be generated by grafting one or more complementarity determining region(s) from a non-human species (e.g., mouse) into a human antibody sequence. The antibody may be partially or completely humanized. See, e.g., Almagro JC, Fransson J. *Humanization of antibodies*. *Front Biosci.* 13:1619-33 (2008) for review of various methods of obtaining humanized antibodies that may be used to obtain a targeting moiety. An antibody may be polyclonal or monoclonal, though monoclonal antibodies are generally preferred. A F(ab')₂ or F(ab') fragment are typically used. Antibodies comprising an Fc domain are also typically used. Methods for producing antibodies that specifically bind to virtually any molecule of interest are known in the art. For example, monoclonal or polyclonal antibodies can be purified from natural sources, e.g., from blood or ascites fluid of an animal that produces the antibody (e.g., following immunization with the molecule or an antigenic fragment thereof) or can be produced recombinantly, in cell culture. Methods of generating antibody fragments, e.g., by digestion, disulfide reduction, or synthesis are known in the art.

[0193] A targeting moiety can be any molecule that specifically binds to a target molecule through a mechanism other than an antigen-antibody interaction. Such a targeting moiety is referred to as a "ligand". For example, a ligand can be a polypeptide, peptide, nucleic acid (e.g., DNA or RNA), carbohydrate, lipid or phospholipid, or small molecule. A small molecule may be an organic compound, whether naturally-occurring or artificially created, that has relatively low molecular weight and is not a protein, polypeptide, nucleic acid, or lipid, typically with a molecular weight of less than about 1500 g/mol and typically having multiple carbon-carbon bonds. In general, an aptamer is an oligonucleotide (e.g., RNA or

DNA, optionally comprising one or more modified nucleosides (e.g., bases or sugars other than the 5 standard bases (A, G, C, T, U) or sugars (ribose and deoxyribose) found most commonly in RNA and DNA), or modified internucleoside linkages (e.g., non-phosphodiester bonds) that, e.g., stabilize the molecule, e.g., by rendering it more resistant to degradation by nucleases) that binds to a particular protein. An oligonucleotide is typically up to about 100 nucleosides long, e.g., between 12 and 100 nucleosides long. Aptamers can be derived using an in vitro evolution process called SELEX, and methods for obtaining aptamers specific for a protein of interest are known in the art. See, e.g., Brody E N, Gold L. J Biotechnol. 2000 March; 74(1):5-13. A peptide nucleic acid or locked nucleic acid may be used.

[0194] A targeting moiety may comprise a peptide. A peptide that binds to a target molecule of interest may be identified using a display technology such as phage display, ribosome display, yeast display, etc.

[0195] Small molecules can be used as ligands. Methods for identifying such ligands are known in the art. For example in vitro screening of small molecule libraries, including combinatorial libraries, and computer-based screening, e.g., to identify small organic compounds that bind to concave surfaces (pockets) of proteins, can identify small molecule ligands for numerous proteins of interest (Huang, Z., Pharm. & Ther. 86: 201-215, 2000).

[0196] Typically, targeting moieties are not proteins or molecules that are typically used as carriers and conjugated to antigens for the purpose of raising antibodies. Examples are carrier proteins or molecules such as bovine serum albumin, keyhole limpet hemocyanin, bovine gamma globulin, and diphtheria toxin. The targeting moiety is typically not an Fc portion of an immunoglobulin molecule. A targeting moiety may be part of a complex comprising one or more additional moieties to which it is covalently or noncovalently attached.

[0197] A target molecule can be any molecule produced by a cell (including any forms expressed on the cell surface or modified forms thereof resulting at least in part from extracellular modification). A target molecule may be an extracellular substance present in or on a tissue. A target molecule may be characteristic of a particular diseased or physiological state or characteristic of one or more cell type(s) or tissue type(s). A target molecule is often a molecule at least partly present at the cell surface (e.g., a transmembrane or otherwise membrane-attached protein) so that at least a portion of the molecule is accessible to binding by an extracellular binding agent such as an antibody. A target molecule may, but need not be, cell type specific. For example, a cell type specific target molecule is often a protein, peptide, mRNA, lipid, or carbohydrate that is present at a higher level on or in a particular cell type or cell type(s) than on or in many other cell types. In some instances a cell type specific target molecule is present at detectable levels only on or in a particular cell type of interest. However, it will be appreciated that a useful cell type specific target molecule need not be absolutely specific for the cell type of interest in order to be considered cell type specific. A cell type specific target molecule for a particular cell type may be expressed at levels at least 3 fold greater in that cell type than in a reference population of cells which may consist, for example, of a mixture containing cells from a plurality (e.g., 5-10 or more) of different tissues or organs in approximately equal amounts. The cell type specific target molecule may be present at levels at least 4-5 fold, between 5-10 fold, or more than 10-fold greater than its average expression in a reference population. Detection or measurement of a cell type specific target molecule may allow one of ordinary skill in the art to distinguish a cell type or types of interest from cells of many, most, or all other types. In general, the presence and/or abundance of most target molecules may be determined using one or more standard techniques such as Northern blotting, in situ hybridization, RT-PCR, sequencing, immunological methods such as immunoblotting, immunodetection (e.g., by immunohistochemistry), or fluorescence detection following staining with fluorescently labeled antibodies (e.g., using FACS), oligonucleotide or cDNA microarray or membrane array, protein microarray analysis, mass spectrometry, etc.

[0198] A target molecule may be a channel, transporter, receptor, or other molecule at least in part exposed at the cell surface. A target molecule may be an anion transporter or water channel (e.g., an aquaporin protein).

[0199] The target molecule may be a protein at least in part exposed at the surface of red blood cells, such as a glycophorin (e.g., glycophorin A, B, C, or D) or band 3.

[0200] The target molecule may be a protein at least in part exposed at the surface of endothelial cells. The target molecule may be present at the surface of normal, healthy vasculature. The target molecule may be present at the surface of activated endothelial cells. The target molecule may be present at the surface of activated endothelial cells but not at the surface of non-activated endothelial cells. A target molecule is typically a molecule whose expression or exposure is induced by a stimulus such as injury or inflammation. A target molecule would usually be recognized as "non-self" by a recipient receiving a transplant containing cells that express the target molecule. The target molecule is sometimes a carbohydrate xenoantigen to which antibodies are commonly found in human beings. Sometimes, the carbohydrate comprises a blood group antigen. Sometimes, the carbohydrate comprises a xenoantigen, e.g., an alpha-gal epitope (Galalpha1-3Galbeta1-(3)4GlcNAc-R) (see, e.g., Macher BA and Galili U. The Galalpha1, 3Galbeta1, 4GlcNAc-R (alpha-Gal) epitope: a carbohydrate of unique evolution and clinical relevance. Biochim Biophys Acta. 1780(2):75-88 (2008)).

[0201] Some disclosed compstatin analogs comprise both a targeting moiety and a CRM

[0202] A targeted compstatin analog may comprise multiple targeting moieties, which can be the same or different. Different targeting moieties may bind to the same target molecule or to different target molecules. A targeted compstatin

analog may be multivalent with respect to the targeting moiety, the compstatin analog, or both.

[0203] A targeted compstatin analog may be produced using methods generally similar to those described in Section VI, wherein a targeting moiety is used instead of, or in addition to, a cell-reactive moiety. A targeted compstatin analog comprising a peptide as a targeting moiety may be synthesized as a polypeptide chain comprising a compstatin analog moiety and a peptide targeting moiety. Optionally, the polypeptide chain comprises one or more spacer peptides between the compstatin analog moiety and the targeting moiety.

[0204] A targeted compstatin analog may have a molar activity of at least about 10%, 20%, or 30%, e.g., between 30% and 40%, between 30% and 50%, between 30% and 60%, between 30% and 70%, between 30% and 80%, between 30% and 90%, or more, of the activity of a corresponding compstatin analog having the same amino acid sequence (and, if applicable, one or more blocking moiety(ies)) but not comprising a targeting moiety. Wherein a targeted compstatin analog comprises multiple compstatin analog moieties, the molar activity of the targeted compstatin analog is at least about 10%, 20%, or 30%, e.g., between 30% and 40%, between 30% and 50%, between 30% and 60%, between 30% and 70%, between 30% and 80%, between 30% and 90%, or more, of the sum of the activities of said compstatin analog moieties.

VIII. Uses

[0205] Cell-reactive, long-acting, or targeted compstatin analogs have a wide variety of uses. Without limiting the invention in any way, certain uses of cell-reactive, long-acting, or targeted compstatin analogs, and related aspects, are described herein. A compstatin analog of the invention may be administered to a subject suffering from or at risk of complement-mediated damage to an organ, tissue, or cells. A cell-reactive compstatin analog may be contacted with an organ, tissue, or cells *ex vivo* and become covalently attached thereto. The organ, tissue, or cells may be introduced into a subject and are protected from damage that would otherwise be caused by the recipient's complement system.

[0206] Compstatin analogs that do not bind covalently to cells can be used for purposes described herein. For example, the compstatin analog of the invention comprises a PEG moiety that increases the lifetime of the compound in the body. Where the discussion below refers to a cell-reactive compstatin analog, the invention provides analogous compositions and uses relating to the compstatin analog of the invention.

[0207] The compstatin analog of the invention can be used for : (1) protecting red blood cells (RBCs) from complement-mediated damage in individuals with disorders such as paroxysmal nocturnal hemoglobinuria or atypical hemolytic uremic syndrome or other disorders characterized by complement-mediated RBC lysis; (2) protecting transplanted organs, tissues, and cells from complement-mediated damage; (3) reducing ischemia/reperfusion (I/R) injury (e.g., in individuals suffering from trauma, vascular obstruction, myocardial infarction, or other situations in which I/R injury may occur); and (4) protecting various body structures (e.g., the retina) or membranes (e.g., synovial membrane) that may be exposed to complement components from complement mediated damage in any of a variety of different complement-mediated disorders. The beneficial effects of inhibiting complement activation at the surface of cells or other body structures are not limited to those resulting directly from protection of the cells or structures themselves against direct complement-mediated damage (e.g., preventing cell lysis). For example, inhibiting complement activation using a cell-reactive compstatin analog may reduce the generation of anaphylotoxins and resulting influx/activation of neutrophils and other pro-inflammatory events and/or reduce potentially damaging release of intracellular contents, thereby potentially having beneficial effects on remote organ systems or throughout the body.

A. Blood cell protection

[0208] In some embodiments of the invention, the compstatin analog of the invention is used to protect blood cells against complement-mediated damage. The blood cells may be any cellular component of the blood, e.g., red blood cells (RBCs), white blood cells (WBCs), and/or platelets. A compstatin analog may be targeted to a target molecule exposed at the cell surface of RBCs such as a glycophorin or band 3. A variety of disorders are associated with complement-mediated damage to blood cells. Such disorders can result, for example, from deficiencies or defects in one or more of an individual's cellular or soluble CRPs, e.g., due to (a) mutation(s) in the gene(s) encoding such proteins; (b) mutation(s) in genes required for production or proper function of one or more CRPs, and/or (c) presence of autoantibodies to one or more CRPs. Complement-mediated RBC lysis can result from the presence of autoantibodies against RBC antigens which may arise due to a diverse set of causes (often being idiopathic). Individuals having such mutation(s) in genes encoding CRPs and/or having antibodies against CRPs or against their own RBCs are at increased risk of disorders involving complement-mediated RBC damage. Individuals who have had one or more episodes characteristic of a disorder are at increased risk of a recurrence.

[0209] Paroxysmal nocturnal hemoglobinuria (PNH) is a relatively rare disorder comprising an acquired hemolytic anemia characterized by complement-mediated intravascular hemolysis, hemoglobinuria, bone marrow failure, and thrombophilia (propensity to develop blood clots). It affects an estimated 16 individuals per million worldwide, occurs in

both sexes, and can arise at any age, frequently striking young adults (Bessler, M. & Hiken, J., Hematology Am Soc Hematol Educ Program, 104-110 (2008); Hillmen, P. Hematology Am Soc Hematol Educ Program, 116-123 (2008)). PNH is a chronic and debilitating disease punctuated by acute hemolytic episodes and results in significant morbidities and reduced life expectancy. In addition to anemia, many patients experience abdominal pain, dysphagia, erectile dysfunction, and pulmonary hypertension, and are at increased risk of renal failure and thromboembolic events.

[0210] PNH was first described as a distinct entity in the 1800s, but it was only in the 1950s, with discovery of the alternative pathway of complement activation, that the cause of hemolysis in PNH was firmly established (Parker C.J. Paroxysmal nocturnal hemoglobinuria: an historical overview. Hematology Am Soc Hematol Educ Program. 93-103 (2008)). CD55 and CD59 are normally attached to the cell membrane via glycosyl phosphatidylinositol (GPI) anchors (glycolipid structures that anchor certain proteins to the plasma membrane). PNH arises as a consequence of nonmalignant clonal expansion of hematopoietic stem cell(s) that have acquired a somatic mutation in the PIGA gene, which encodes a protein involved in synthesis of GPI anchors (Takeda J, et al. Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria. Cell. 73:703-711 (1993)). Progeny of such stem cells are deficient in GPI-anchored proteins, including CD55 and CD59. This defect renders these cells susceptible to complement-mediated RBC lysis. Flow cytometric analysis using antibodies to GPI-anchored proteins is often used for diagnosis. It detects deficiency of GPI-anchored proteins at the cell surface and allows determination of the degree of deficiency and the proportion of affected cells (Brodsky RA. Advances in the diagnosis and therapy of paroxysmal nocturnal hemoglobinuria. Blood Rev. 22(2):65-74 (2008)). PNH type III RBCs are completely deficient in GPI-linked proteins and are highly sensitive to complement whereas PNH type II RBCs have a partial deficiency and are less sensitive. FLAER is a fluorescently labeled inactive variant of proaerolysin (a bacterial toxin that binds GPI anchors) and is increasingly used together with flow cytometry for diagnosis of PNH. Lack of binding of FLAER to granulocytes is sufficient for diagnosis of PNH. Cell-reactive compstatin analogs may protect PNH RBCs from deposition of C3b.

[0211] A compstatin analog may be administered to a subject suffering from atypical hemolytic syndrome (aHUS). aHUS is a chronic disorder characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure and is caused by inappropriate complement activation, often due to mutations in genes encoding complement regulatory proteins (Warwicker, P., et al.. Kidney Int 53, 836-844 (1998); Kavanagh, D. & Goodship, T. Pediatr Nephrol 25, 2431-2442 (2010)). Mutations in the complement factor H (CFH) gene are the most common genetic abnormality in patients with aHUS, and 60-70% of these patients die or reach end stage renal failure within one year after disease onset (Kavanagh & Goodship, supra.) Mutations in factor I, factor B, C3, factor H-related proteins 1-5, and thrombomodulin have also been described. Other causes of aHUS include autoantibodies against complement regulatory proteins such as CFH. A compstatin analog may be administered to a subject that has been identified as having a mutation in factor I, factor B, C3, factor H-related proteins 1-5, or thrombomodulin or has been identified as having antibodies against a complement regulatory protein, e.g., CFH.

[0212] Complement-mediated hemolysis occurs in a diverse group of other conditions including autoimmune hemolytic anemias that involve antibodies that bind to RBCs and lead to complement-mediated hemolysis. For example, such hemolysis can occur in primary chronic cold agglutinin disease and certain reactions to drugs and other foreign substances (Berentsen, S., et al., Hematology 12, 361-370 (2007); Rosse, W.F., Hillmen, P. & Schreiber, A.D. Hematology Am Soc Hematol Educ Program, 48-62 (2004)). A compstatin analog may be administered to a subject suffering from or at risk of chronic cold agglutinin disease. The invention therefore provides a compstatin analog of the invention for use in treating a subject suffering from or at risk of the HELLP syndrome, which is defined by the existence of hemolysis, elevated liver enzymes, and low platelet count and is associated with mutations in complement regulatory protein(s) in at least some subjects (Fakhouri, F., et al., 112: 4542-4545 (2008)).

[0213] Cell-reactive compstatin analogs may be used to protect RBCs or other cellular components of blood to be transfused into a subject. Certain examples of such uses are discussed further in below. As noted above, targeted and/or long-acting compstatin analogs can be used to inhibit complement-mediated hemolysis and/or RBC damage. A long-acting compstatin analog comprising a (CH₂CH₂O) moiety may be used to treat PNH or aHUS.

B. Transplantation

[0214] Transplantation is a therapeutic approach of increasing importance, providing a means to replace organs and tissues that have been damaged through trauma, disease, or other conditions. Kidneys, liver, lungs, pancreas, and heart are among the organs that can be successfully transplanted. Tissues that are frequently transplanted include bones, cartilage, tendons, cornea, skin, heart valves, and blood vessels. Pancreatic islet or islet cell transplantation is a promising approach for treatment of diabetes, e.g., type I diabetes. For purposes of the invention, an organ, tissue, or cell (or population of cells) that is to be transplanted, is being transplanted, or has been transplanted may be referred to as a "graft". For purposes hereof, a blood transfusion is considered a "graft".

[0215] Transplantation subjects the graft to a variety of damaging events and stimuli that can contribute to graft dysfunction and, potentially, failure. For example, ischemia-reperfusion (I/R) injury is a common and significant cause

of morbidity and mortality in the case of many grafts (particularly solid organs) and can be a major determinant of likelihood of graft survival. Transplant rejection is one of the major risks associated with transplants between genetically different individuals and can lead to graft failure and a need to remove the graft from the recipient.

[0216] The compstatin analog of the invention may be used to protect a graft from complement-mediated damage. A cell-reactive compstatin analog reacts with cells of the graft, becomes covalently attached thereto, and inhibits complement activation. A cell-targeted compstatin analog binds to a target molecule in the graft (e.g., expressed by endothelial cells or other cells in the graft) and inhibits complement activation. A target molecule may be, e.g., is a molecule whose expression is induced or stimulated by a stimulus such as injury or inflammation, molecule that would be recognized as "non-self" by the recipient, a carbohydrate xenoantigen to which antibodies are commonly found in human beings such as a blood group antigen or a xenoantigen, e.g., a molecule comprising an alpha-gal epitope. In some embodiments, a reduction in complement activation can be demonstrated by a reduction in average C4d deposition in blood vessels of grafts that have been contacted with a compstatin analog, e.g., a cell-reactive compstatin analog, as compared with the average level of C4d deposition in grafts that have not been contacted with a compstatin analog (e.g., in subjects who are matched with respect to the grafts and other therapy that they receive).

[0217] A graft can be contacted with a cell-reactive, long-acting, or targeted compstatin analog prior to, during, and/or after being transplanted. For example, prior to transplantation a graft removed from a donor can be contacted with a liquid comprising a cell-reactive, long-acting, or targeted compstatin analog. For example, the graft can be bathed in and/or perfused with the solution. A cell-reactive, long-acting, or targeted compstatin analog may be administered to a donor prior to removal of the graft. A cell-reactive, long-acting, or targeted compstatin analog may be administered to a recipient during and/or after the introduction of the graft. A cell-reactive compstatin, long-acting, or targeted analog may be delivered locally to the transplanted graft. A cell-reactive compstatin analog may be administered systemically, e.g., intravenously. Disclosed herein is a composition comprising: (a) an isolated graft; and (b) a cell-reactive, long-acting, or targeted compstatin analog. The disclosed composition may further comprise a liquid solution suitable for contacting (e.g., suitable for rinsing, washing, bathing, perfusing, maintaining, or storing) a graft (e.g., an organ) such as an isolated graft that has been removed from a donor and is awaiting transplantation to a recipient. Disclosed herein is a composition comprising: (a) a liquid solution suitable for contacting a graft (e.g., an organ); and (b) a cell-reactive, long-acting, or targeted compstatin analog. The liquid solution can be any liquid solution that is physiologically acceptable to the graft (e.g., appropriate osmotic composition, non-cytotoxic) and medically acceptable in view of the subsequent introduction of the graft into the recipient (e.g., preferably sterile or at least reasonably free from microorganisms or other contaminants) and compatible with the cell-reactive compstatin analog (i.e., will not destroy the reactivity of the compstatin analog) or compatible with the long-acting or targeted compstatin analog. The solution may be any solution known in the art for any such purposes, for example Marshall's or Hyperosmolar Citrate (Soltran®, Baxter Healthcare), University of Wisconsin (UW) solution (ViaSpan™, Bristol Myers Squibb), Histidine Tryptophan Ketoglutarate (HTK) solution (Custodial®, Kohler Medical Limited), EuroCollins (Fresenius), and Celsior® (Sangstat Medical), Polysol, IGL-1, or AQIX® RS-1. Of course other solutions, e.g., containing equivalent or similar ingredients in the same or different concentrations could be used within the scope of physiologically acceptable compositions. A solution typically does not contain ingredient(s) with which the cell-reactive compstatin analog would be expected to significantly react, and any solution may be modified or designed to lack such ingredients. The cell-reactive compstatin analog may be present in the graft-compatible solution at a concentration of, e.g., between 0.01 mg/ml and 100 mg/ml or may be added to the solution to achieve such concentration.

[0218] Also disclosed is a kit comprising: (a) a cell-reactive, long-acting, or targeted compstatin analog; and (b) a graft-compatible solution or solid (e.g., powder) components thereof. The cell-reactive, long-acting, or targeted compstatin analog may be provided in solid form (e.g., powder) or at least in part dissolved in a solution. The cell-reactive, long-acting, or targeted compstatin analog and/or graft-compatible solution may be provided in predetermined amounts, so that when combined, a solution of appropriate concentration for contacting a graft with the cell-reactive, long-acting, or targeted compstatin analog is produced. The cell-reactive, long-acting, or targeted compstatin analog and graft-compatible solution or solid (e.g., powder) components thereof may be in separate containers within the kit. The cell-reactive compstatin analog and components of a graft-compatible solution may be both provided in solid (e.g., powder) form, either in separate containers or mixed. The kit may comprise instructions for use, e.g., instructions for adding a cell-reactive, long-acting, or targeted compstatin analog to a graft-compatible solution and/or instructions for contacting a graft with a cell-reactive compstatin analog. Optionally the kit contains a label approved by a government agency responsible for regulating products used in transplantation, cell therapy, and/or blood transfusion.

[0219] Also disclosed herein is a compstatin analog for use in covalent attachment to an isolated graft, the use comprising contacting the isolated graft with a cell-reactive compstatin analog. Also disclosed herein is an isolated graft having a compstatin analog covalently attached thereto. Typically the isolated graft has many molecules of compstatin analog attached thereto. In some disclosed aspects, a graft is or comprises a solid organ such as a kidney, liver, lung, pancreas, or heart. In some disclosed aspects, a graft is or comprises bone, cartilage, fascia, tendon, ligament, cornea, sclera, pericardium, skin, heart valve, blood vessel, amniotic membrane, or dura mater. In some disclosed aspects, a graft comprises multiple organs such as a heart-lung or pancreas-kidney graft. In some disclosed aspects, a graft

comprises less than a complete organ or tissue. For example, a graft may contain a portion of an organ or tissue, e.g., a liver lobe, section of blood vessel, skin flap, or heart valve. In some disclosed aspects, a graft comprises a preparation comprising isolated cells or tissue fragments that have been isolated from their tissue of origin but retain at least some tissue architecture, e.g., pancreatic islets. In some disclosed aspects, a preparation comprises isolated cells that are not attached to each other via connective tissue, e.g., hematopoietic stem cells or progenitor cells derived from peripheral and/or cord blood, or whole blood or any cell-containing blood product such as red blood cells (RBCs) or platelets. In some disclosed aspects a graft is obtained from a deceased donor (e.g., a "donation after brain death" (DBD) donor or "donation after cardiac death" donor). In some disclosed aspects, depending on the particular type of graft, a graft is obtained from a living donor. For example, kidneys, liver sections, blood cells, are among the types of grafts that can often be obtained from a living donor without undue risk to the donor and consistent with sound medical practice.

[0220] In some disclosed aspects, a graft is a xenograft (i.e., the donor and recipient are of different species). In some disclosed aspects a graft is an autograft (i.e., a graft from one part of the body to another part of the body in the same individual). In some disclosed aspects, a graft is an isograft (i.e., the donor and recipient are genetically identical). In most disclosed aspects, the graft is an allograft (i.e., the donor and recipient are genetically non-identical members of the same species). In the case of an allograft, the donor and recipient may or may not be genetically related (e.g., family members). Typically, the donor and recipient have compatible blood groups (at least ABO compatibility and optionally Rh, Kell and/or other blood cell antigen compatibility). The recipient's blood may have been screened for alloantibodies to the graft and/or the recipient and donor since the presence of such antibodies can lead to hyperacute rejection (i.e., rejection beginning almost immediately, e.g., within several minutes after the graft comes into contact with the recipient's blood). A complement-dependent cytotoxicity (CDC) assay can be used to screen a subject's serum for anti-HLA antibodies. The serum is incubated with a panel of lymphocytes of known HLA phenotype. If the serum contains antibodies against HLA molecules on the target cells, cell death due to complement-mediated lysis occurs. Using a selected panel of target cells allows one to assign specificity to the detected antibody. Other techniques useful for determining the presence or absence anti-HLA antibodies and, optionally, determining their HLA specificity, include ELISA assays, flow cytometry assays, microbead array technology (e.g., Luminex technology). The methodology for performing these assays is well known, and a variety of kits for performing them are commercially available.

[0221] A cell-reactive, long-acting, or targeted compstatin analog may inhibit complement-mediated rejection. For example, a cell-reactive, long-acting, or targeted compstatin analog may be used to inhibit hyperacute rejection. Hyperacute rejection is caused at least in part by antibody-mediated activation of the recipient's complement system via the classical pathway and resulting MAC deposition on the graft. It typically results from the presence in the recipient of pre-existing antibodies that react with the graft. While it is desirable to attempt to avoid hyperacute rejection by appropriate matching prior to transplantation, it may not always possible to do so due, e.g., to time and/or resource constraints. Furthermore, some recipients (e.g., multiply transfused individuals, individuals who have previously received transplants, women who have had multiple pregnancies) may already have so many pre-formed antibodies, potentially including antibodies to antigens that are not typically tested for, that it can be difficult or perhaps almost impossible to obtain with confidence a compatible graft in a timely manner. Such individuals are at increased risk of hyperacute rejection.

[0222] A cell-reactive, long-acting, or targeted compstatin analog may be used to inhibit acute rejection or graft failure. As used herein, "acute rejection" refers to rejection occurring between at least 24 hours, typically at least several days to a week, after a transplant, up to 6 months after the transplant. Acute antibody-mediated rejection (AMR) often involves an acute rise in donor-specific alloantibody (DSA) in the first few weeks after transplantation. Without wishing to be bound by any theory, it is possible that pre-existing plasma cells and/or the conversion of memory B cells to new plasma cells play a role in the increased DSA production. Such antibodies can result in complement-mediated damage to the graft, which can be inhibited by contacting the graft with a cell-reactive compstatin analog. Without wishing to be bound by any theory, inhibiting complement activation at the graft may reduce leukocyte (e.g., neutrophil) infiltration, another contributor to acute graft failure.

[0223] A cell-reactive, long-acting, or targeted compstatin analog may be used to inhibit complement-mediated I/R injury to a graft. As discussed further below, I/R injury can occur upon reperfusion of tissue whose blood supply has been temporarily disrupted, as occurs in transplanted organs. Reducing I/R injury would reduce the likelihood of acute graft dysfunction or reduce its severity, and reduce the likelihood of acute graft failure.

[0224] A cell-reactive, long-acting, or targeted compstatin analog may be used to inhibit chronic rejection and/or chronic graft failure. As used herein, "chronic rejection or graft failure" refers to rejection or failure occurring at least 6 months post-transplant, e.g., between 6 months and 1, 2, 3, 4, 5 years, or more post-transplant, often after months to years of good graft function. It is caused by a chronic inflammatory and immune response against the graft. For purposes hereof, chronic rejection can include chronic allograft vasculopathy, a term used to refer to fibrosis of the internal blood vessels of the transplanted tissue. As immunosuppressive regimens have reduced the incidence of acute rejection, chronic rejection is becoming more prominent as a cause of graft dysfunction and failure. There is increasing evidence that B-cell production of alloantibody is an important element in the genesis of chronic rejection and graft failure (Kwun J. and Knechtle SJ, Transplantation, 88(8):955-61 (2009). Earlier damage to the graft may be a contributing factor leading to

chronic processes such as fibrosis that can ultimately lead to chronic rejection. Thus, inhibiting such earlier damage using a cell-reactive compstatin analog may delay and/or reduce the likelihood or severity of chronic graft rejection.

[0225] Provided herein is a long-acting compstatin analog for use in administration to a graft recipient to inhibit graft rejection and/or graft failure.

C. Ischemia/Reperfusion Injury

[0226] Ischemia-reperfusion (I/R) injury is an important cause of tissue damage following trauma and in other conditions associated with temporary disruption of blood flow such as myocardial infarction, stroke, severe infection, vascular disease, aneurysm repair, cardiopulmonary bypass, and transplantation.

[0227] In the setting of trauma, systemic hypoxemia, hypotension, and local interruption of the blood supply resulting from contusions, compartment syndrome, and vascular injuries cause ischemia that damages metabolically active tissues. Restoration of the blood supply triggers an intense systemic inflammatory reaction that is often more harmful than the ischemia itself. Once the ischemic region is reperfused, factors that are produced and released locally enter the circulatory system and reach remote locations, sometimes causing significant damage to organs not affected by the original ischemic insult, such as the lungs and intestine, leading to single and multiple organ dysfunction. Complement activation occurs soon after reperfusion and is a key mediator of post-ischemic damage, both directly and through its chemoattractive and stimulatory effects on neutrophils. All three major complement pathways are activated and, acting cooperatively or independently, are involved in I/R related adverse events affecting numerous organ systems. The invention therefore provides a compstatin analog of the invention for use in treating trauma in a subject who has recently (e.g., within the preceding 2, 4, 8, 12, 24, or 48 hours) experienced trauma, e.g., trauma that puts the subject at risk of I/R injury, e.g., due to systemic hypoxemia, hypotension, and/or local interruption of the blood supply. The cell-reactive compstatin analog may be administered intravascularly, optionally into a blood vessel that supplies an injured body part or directly to the body part. The subject may be suffering from spinal cord injury, traumatic brain injury, burn, and/or hemorrhagic shock.

[0228] Provided herein is a compstatin analog of the invention for use in administration to a subject prior to, during, or after a surgical procedure, e.g., a surgical procedure that is expected to temporarily disrupt blood flow to a tissue, organ, or portion of the body. Examples of such procedures include cardiopulmonary bypass, angioplasty, heart valve repair/replacement, aneurysm repair, or other vascular surgeries. The cell-reactive compstatin analog may be administered prior to, after, and/or during an overlapping time period with the surgical procedure.

[0229] Provided herein is a compstatin analog of the invention for use in treating an MI, thromboembolic stroke, deep vein thrombosis, or pulmonary embolism in a subject, optionally in combination with a thrombolytic agent such as tissue plasminogen activator (tPA) (e.g., alteplase (Activase), reteplase (Retavase), tenecteplase (TNKase)), anistreplase (Eminase), streptokinase (Kabikinase, Streptase), or urokinase (Abbokinase). The compstatin analog may be administered prior to, after, and/or during an overlapping time period with the thrombolytic agent.

[0230] Provided herein is a compstatin analog of the invention for use in treating I/R injury.

D. Other Complement-Mediated Disorders

[0231] Provided herein is a compstatin analog of the invention for use in treatment of an eye disorder such as age-related macular degeneration (AMD), diabetic retinopathy, glaucoma, or uveitis by introduction of the compstatin analog into the eye. For example, a cell-reactive compstatin analog may be introduced into the vitreous cavity (e.g., by intravitreal injection), for use in treatment of a subject at suffering from or at risk of AMD. A compstatin analog may be introduced into the anterior chamber, e.g., to treat anterior uveitis.

[0232] Also provided is a compstatin analog of the invention for use in treating a subject suffering from or at risk of an autoimmune disease, e.g., an autoimmune disease mediated at least in part by antibodies against one or more self antigens.

[0233] Also provided is a compstatin analog of the invention for use in treating arthritis (e.g., rheumatoid arthritis) in a subject in need thereof. The compstatin analog may be introduced into the synovial cavity of the subject. Of course it may be administered systemically.

[0234] Also provided is a compstatin analog of the invention for use in treating a subject suffering from or at risk of an intracerebral hemorrhage.

[0235] Also provided is a compstatin analog for use in treating a subject suffering from or at risk of myasthenia gravis.

[0236] Also provided is a compstatin analog of the invention for use in treating a subject suffering from or at risk of neuromyelitis optica (NMO),

[0237] Also provided is a compstatin analog of the invention for use in treating a subject suffering from or at risk of membranoproliferative glomerulitis (MPGN), e.g., MPGN type I, MPGN type II, or MPGN type III.

[0238] Also provided is a compstatin analog of the invention for use in treating a subject suffering from or at risk of a

neurodegenerative disease. Also provided is a compstatin analog of the invention for use in treating a subject suffering from neuropathic pain or at risk of developing neuropathic pain. Also provided is a compstatin analog of the invention for use in treating a subject suffering from or at risk of rhinosinusitis or nasal polyposis. Also provided is a compstatin analog of the invention for use in treating a subject suffering from or at risk of cancer. Also provided is a compstatin analog of the invention for use in treating a subject suffering from or at risk of sepsis. Also provided is a compstatin analog of the invention for use in treating a subject suffering from or at risk of adult respiratory distress syndrome.

[0239] Also provided is a compstatin analog of the invention for use in treating subject suffering from or at risk of anaphylaxis or infusion reaction. For example, the compstatin analog may be administered to the subject prior to, during, or after receiving a drug or a vehicle that may cause anaphylaxis or infusion reaction. Also provided is a compstatin analog of the invention for use in treating a subject at risk of or suffering from anaphylaxis from a food (e.g., peanut, shellfish, or other food allergens), insect sting (e.g., bee, wasp).

[0240] The compstatin analog may be administered locally or systemically.

[0241] Also provided is a compstatin analog of the invention for use in treating a respiratory disease, e.g., asthma or chronic obstructive pulmonary disease (COPD). The compstatin analog may, for example, be administered to the respiratory tract by inhalation, e.g., as a dry powder or via nebulization, or may be administered by injection, e.g., intravenously, intramuscularly, or subcutaneously. Also provided is a compstatin analog of the invention for use in treating severe asthma, e.g., asthma that is not sufficiently controlled by bronchodilators and/or inhaled corticosteroids.

[0242] Also provided is a compstatin analog of the invention for use in treating a complement-mediated disorder, e.g., a chronic complement-mediated disorder in a subject in need thereof. Also provided is a compstatin analog of the invention for use in treating a Th17-associated disorder in a subject in need thereof.

[0243] A "chronic disorder" may be a disorder that persists for at least 3 months and/or is accepted in the art as being a chronic disorder. A chronic disorder may persist for at least 6 months, e.g., at least 1 year, or more, e.g., indefinitely. One of ordinary skill in the art will appreciate that at least some manifestations of various chronic disorders may be intermittent and/or may wax and wane in severity over time. A chronic disorder may be progressive, e.g., having a tendency to become more severe or affect larger areas over time. A number of chronic complement-mediated disorders are discussed herein. A chronic complement-mediated disorder may be any chronic disorder in which complement activation (e.g., excessive or inappropriate complement activation) is involved, e.g., as a contributing and/or at least partially causative factor. For convenience, disorders are sometimes grouped by reference to an organ or system that is often particularly affected in subjects suffering from the disorder. It will be appreciated that a number of disorders can affect multiple organs or systems, and such classification(s) are in no way limiting. Furthermore, a number of manifestations (e.g., symptoms) may occur in subjects suffering from any of a number of different disorders. Non-limiting information regarding disorders of interest herein may be found, e.g., in standard textbooks of internal medicine such as Cecil Textbook of Medicine (e.g., 23rd edition), Harrison's Principles of Internal Medicine (e.g., 17th edition), and/or standard textbooks focusing on particular areas of medicine, particular body systems or organs, and/or particular disorders.

[0244] A chronic complement-mediated disorder may be a Th2-associated disorder. As used herein, a Th2-associated disorder is a disorder characterized by an excessive number and/or excessive or inappropriate activity of CD4⁺ helper T cells of the Th2 subtype ("Th2 cells") in the body or a portion thereof, e.g., in at least one tissue, organ, or structure. For example, there may be a predominance of Th2 cells relative to CD4⁺ helper T cells of the Th1 subtype ("Th1 cells") e.g., in at least one tissue, organ, or structure affected by a disorder. As known in the art, Th2 cells typically secrete characteristic cytokines such as interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13), while Th1 cells typically secrete interferon- γ (IFN- γ) and tumor necrosis factor β (TNF β). A Th2-associated disorder may be characterized by excessive production and/or amount of IL-4, IL-5, and/or IL-13, e.g., relative to IFN- γ and/or TNF β e.g., in at least some at least one tissue, organ, or structure.

[0245] A chronic complement-mediated disorder may be a Th17-associated disorder. As described in further detail in PCT/US2012/043845, filed June 22, 2012, entitled "Methods of Treating Chronic Disorders with Complement Inhibitors", complement activation and Th17 cells participate in a cycle that involves dendritic cells and antibodies and that contributes to maintenance of a pathologic immunologic microenvironment underlying a range of disorders. Without wishing to be bound by any theory, the pathologic immunologic microenvironment, once established, is self-sustaining and contributes to cell and tissue injury. A long-acting compstatin analogs may be used to treat Th17-associated disorders.

[0246] As used herein, a Th17-associated disorder is a disorder characterized by an excessive number and/or excessive or inappropriate activity of CD4⁺ helper T cells of the Th17 subtype ("Th17 cells") in the body or a portion thereof, e.g., in at least one tissue, organ, or structure. For example, there may be a predominance of Th17 cells relative to Th1 and/or Th2 cells, e.g., in at least one tissue, organ, or structure affected by a disorder. A predominance of Th17 cells may be a relative predominance, e.g., the ratio of Th17 cells to Th1 cells and/or the ratio of Th17 cells to Th2 cells, is increased relative to normal values. The ratio of Th17 cells to T regulatory cells (CD4⁺CD25⁺ regulatory T cells, also termed "Treg cells"), may be increased relative to normal values. Formation of Th17 cells and/or activation of Th17 cells is promoted by various cytokines, e.g., interleukin 6 (IL-6), interleukin 21 (IL-21), interleukin 23 (IL-23), and/or interleukin

1 β (IL-1 β). Formation of Th17 cells encompasses differentiation of precursor T cells, e.g., naive CD4⁺ T cells, towards a Th17 phenotype and their maturation into functional Th17 cells. Formation of Th17 cells may encompass any aspect of development, proliferation (expansion), survival, and/or maturation of Th17 cells. A Th17-associated disorder may be characterized by excessive production and/or amount of IL-6, IL-21, IL-23, and/or IL-1 β . Th17 cells typically secrete characteristic cytokines such as interleukin-17A (IL-17A), interleukin-17F (IL-17F), interleukin-21 (IL-21), and interleukin-22 (IL-22). A Th17-associated disorder may be characterized by excessive production and/or amount of a Th17 effector cytokine, e.g., IL-17A, IL-17F, IL-21, and/or IL-22. Excessive production or amount of a cytokine may be detectable in the blood. Excessive production or amount of a cytokine may be detectable locally, e.g., in at least one tissue, organ or structure. A Th17-associated disorder may be associated with a decreased number of Tregs and/or decreased amount of a Treg-associated cytokine. A Th17 disorder may be any chronic inflammatory disease, which term encompasses a range of ailments characterized by self-perpetuating immune insults to a variety of tissues and that seem to be dissociated from the initial insult that caused the ailment (which may be unknown). A Th17-associated disorder may be any autoimmune disease. Many if not most "chronic inflammatory diseases" may in fact be auto-immune diseases. Examples of Th17-associated disorders include inflammatory skin diseases such as psoriasis and atopic dermatitis; systemic scleroderma and sclerosis; inflammatory bowel disease (IBD) (such as Crohn's disease and ulcerative colitis); Behcet's Disease; dermatomyositis; polymyositis; multiple sclerosis (MS); dermatitis; meningitis; encephalitis; uveitis; osteoarthritis; lupus nephritis; rheumatoid arthritis (RA), Sjorgen's syndrome, multiple sclerosis, vasculitis; central nervous system (CNS) inflammatory disorders, chronic hepatitis; chronic pancreatitis, glomerulonephritis; sarcoidosis; thyroiditis, pathologic immune responses to tissue/organ transplantation (e.g., transplant rejection); COPD, asthma, bronchiolitis, hypersensitivity pneumonitis, idiopathic pulmonary fibrosis (IPF), periodontitis, and gingivitis. A Th17 disease may be a classically known auto-immune disease such as Type I diabetes or psoriasis. A Th17-associated disorder may be age-related macular degeneration.

[0247] A chronic complement-mediated disorder may be an IgE-associated disorder. As used herein, an "IgE-associated disorder" is a disorder characterized by excessive and/or inappropriate production and/or amount of IgE, excessive or inappropriate activity of IgE producing cells (e.g., IgE producing B cells or plasma cells), and/or excessive and/or inappropriate activity of IgE responsive cells such as eosinophils or mast cells. An IgE-associated disorder may be characterized by elevated levels of total IgE and/or allergen-specific IgE, in the plasma of a subject and/or locally.

[0248] A chronic complement-mediated disorder may be characterized by the presence of autoantibodies and/or immune complexes in the body, which may activate complement via, e.g., the classical pathway. Autoantibodies may, for example, bind to self antigens, e.g., on cells or tissues in the body. Autoantibodies may bind to antigens in blood vessels, skin, nerves, muscle, connective tissue, heart, kidney, thyroid, etc. A chronic complement-mediated disorder may not be characterized by autoantibodies and/or immune complexes.

[0249] A chronic complement-mediated disorder may be a respiratory disorder. A chronic respiratory disorder may be asthma or chronic obstructive pulmonary disease (COPD). A chronic respiratory disorder may be pulmonary fibrosis (e.g., idiopathic pulmonary fibrosis), radiation-induced lung injury, allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis (also known as allergic alveolitis), eosinophilic pneumonia, interstitial pneumonia, sarcoid, Wegener's granulomatosis, or bronchiolitis obliterans. Provided herein is a compstatin analog of the invention for use in treating a chronic respiratory disorder, e.g., asthma, COPD, pulmonary fibrosis, radiation-induced lung injury, allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis (also known as allergic alveolitis), eosinophilic pneumonia, interstitial pneumonia, sarcoid, Wegener's granulomatosis, or bronchiolitis obliterans in a subject in need thereof.

[0250] A chronic complement-mediated disorder may be allergic rhinitis, rhinosinusitis, or nasal polyposis. Provided herein is a compstatin analog of the invention for use in treating allergic rhinitis, rhinosinusitis, or nasal polyposis in a subject in need thereof.

[0251] A chronic complement-mediated disorder may be a disorder that affects the musculoskeletal system. Examples of such disorders include inflammatory joint conditions (e.g., arthritis such as rheumatoid arthritis or psoriatic arthritis, juvenile chronic arthritis, spondyloarthropathies Reiter's syndrome, gout). A musculoskeletal system disorder may result in symptoms such as pain, stiffness and/or limitation of motion of the affected body part(s). Inflammatory myopathies include dermatomyositis, polymyositis, and various others are disorders of chronic muscle inflammation of unknown etiology that result in muscle weakness. A chronic complement-mediated disorder may be myasthenia gravis. Provided herein is a compstatin analog of the invention for use in treating any of the foregoing disorders affecting the musculoskeletal system in a subject in need thereof.

[0252] A chronic complement-mediated disorder may be a disorder that affects the integumentary system. Examples of such disorders include, e.g., atopic dermatitis, psoriasis, pemphigus, systemic lupus erythematosus, dermatomyositis, scleroderma, sclerodermatomyositis, Sjogren syndrome, and chronic urticaria. Provided herein is a compstatin analog of the invention for use in treating any of the foregoing disorders affecting the integumentary system in a subject in need thereof.

[0253] A chronic complement-mediated disorder may affect the nervous system, e.g., the central nervous system (CNS) and/or peripheral nervous system (PNS). Examples of such disorders include, e.g., multiple sclerosis, other

chronic demyelinating diseases, amyotrophic lateral sclerosis, chronic pain, stroke, allergic neuritis, Huntington's disease, Alzheimer's disease, and Parkinson's disease. Provided herein is a compstatin analog of the invention for use in treating any of the foregoing disorders affecting the nervous system in a subject in need thereof, the use comprising administering the complement inhibitor according to a dosing schedule described herein to the subject.

[0254] A chronic complement-mediated disorder may affect the circulatory system. For example, the disorder may be a vasculitis or other disorder associated with vessel inflammation, e.g., blood vessel and/or lymph vessel inflammation. A vasculitis may be polyarteritis nodosa, Wegener's granulomatosis, giant cell arteritis, Churg-Strauss syndrome, microscopic polyangiitis, Henoch-Schonlein purpura, Takayasu's arteritis, Kawasaki disease, or Behcet's disease. A subject, e.g., a subject in need of treatment for vasculitis, may be positive for antineutrophil cytoplasmic antibody (ANCA).

[0255] A chronic complement-mediated disorder may affect the gastrointestinal system. For example, the disorder may be inflammatory bowel disease, e.g., Crohn's disease or ulcerative colitis. Provided herein is a compstatin analog of the invention for use in treating a chronic complement-mediated disorder that affects the gastrointestinal system in a subject in need thereof.

[0256] A chronic complement-mediated disorder may be a thyroiditis (e.g., Hashimoto's thyroiditis, Graves' disease, post-partum thyroiditis), myocarditis, hepatitis (e.g., hepatitis C), pancreatitis, glomerulonephritis (e.g., membranoproliferative glomerulonephritis or membranous glomerulonephritis), or panniculitis.

[0257] Provided herein is a compstatin analog of the invention for use in treating chronic pain in a subject in need thereof. A subject may suffer from neuropathic pain. Neuropathic pain has been defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system, in particular, pain arising as a direct consequence of a lesion or disease affecting the somatosensory system. For example, neuropathic pain may arise from lesions that involve the somatosensory pathways with damage to small fibres in peripheral nerves and/or to the spino-thalamocortical system in the CNS. Neuropathic pain may arise from autoimmune disease (e.g., multiple sclerosis), metabolic disease (e.g., diabetes), infection (e.g., viral disease such as shingles or HIV), vascular disease (e.g., stroke), trauma (e.g., injury, surgery), or cancer. For example, neuropathic pain can be pain that persists after healing of an injury or after cessation of a stimulus of peripheral nerve endings or pain that arises due to damage to nerves. Exemplary conditions of or associated with neuropathic pain include painful diabetic neuropathy, post-herpetic neuralgia (e.g., pain persisting or recurring at the site of acute herpes zoster 3 or more months after the acute episode), trigeminal neuralgia, cancer related neuropathic pain, chemotherapy-associated neuropathic pain, HIV-related neuropathic pain (e.g., from HIV neuropathy), central/post-stroke neuropathic pain, neuropathy associated with back pain, e.g., low back pain (e.g., from radiculopathy such as spinal root compression, e.g., lumbar root compression, which compression may arise due to disc herniation), spinal stenosis, peripheral nerve injury pain, phantom limb pain, polyneuropathy, spinal cord injury related pain, myelopathy, and multiple sclerosis. The complement inhibitor may be administered according to an inventive dosing schedule to treat neuropathic pain in a subject with one or more of the afore-mentioned conditions.

[0258] A chronic complement-mediated disorder may be a chronic eye disorder. The chronic eye disorder may be characterized by macular degeneration, choroidal neovascularization (CNV), retinal neovascularization (RNV), ocular inflammation, or any combination of the foregoing. Macular degeneration, CNV, RNV, and/or ocular inflammation may be a defining and/or diagnostic feature of the disorder. Exemplary disorders that are characterized by one or more of these features include, but are not limited to, macular degeneration related conditions, diabetic retinopathy, retinopathy of prematurity, proliferative vitreoretinopathy, uveitis, keratitis, conjunctivitis, and scleritis. Macular degeneration related conditions include, e.g., age-related macular degeneration (AMD). A subject may be in need of treatment for wet AMD. A subject may be in need of treatment for dry AMD. A subject may be in need of treatment for geographic atrophy (GA). A subject may be in need of treatment for ocular inflammation. Ocular inflammation can affect a large number of eye structures such as the conjunctiva (conjunctivitis), cornea (keratitis), episclera, sclera (scleritis), uveal tract, retina, vasculature, and/or optic nerve. Evidence of ocular inflammation can include the presence of inflammation-associated cells such as white blood cells (e.g., neutrophils, macrophages) in the eye, the presence of endogenous inflammatory mediator(s), one or more symptoms such as eye pain, redness, light sensitivity, blurred vision and floaters, etc. Uveitis is a general term that refers to inflammation in the uvea of the eye, e.g., in any of the structures of the uvea, including the iris, ciliary body or choroid. Specific types of uveitis include iritis, iridocyclitis, cyclitis, pars planitis and choroiditis. A subject may be in need of treatment for geographic atrophy (GA). The chronic eye disorder may be an eye disorder characterized by optic nerve damage (e.g., optic nerve degeneration), such as glaucoma.

[0259] As noted above, the chronic respiratory disease may be asthma. Information regarding risk factors, epidemiology, pathogenesis, diagnosis, current management of asthma, etc., may be found, e.g., in "Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma". National Heart Lung and Blood Institute. 2007. <http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.pdf>. ("NHLBI Guidelines"; www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm), Global Initiative for Asthma, Global Strategy for Asthma Management and Prevention 2010 "GINA Report") and/or standard textbooks of internal medicine such as Cecil Textbook of Medicine (20th edition), Harrison's Principles of Internal Medicine (17th edition), and/or standard textbooks focusing on pulmonary medicine. Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, such as, mast cells, eosinophils,

T lymphocytes, macrophages, neutrophils, and epithelial cells. Asthmatic individuals experience recurrent episodes associated with symptoms such as wheezing, breathlessness (also termed dyspnea or shortness of breath), chest tightness, and coughing. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible, either spontaneously or with treatment. The inflammation also causes an associated increase in the existing bronchial hyperresponsiveness to a variety of stimuli. Airway hyperresponsiveness (an exaggerated bronchoconstrictor response to stimuli) is a typical feature of asthma. In general, airflow limitation results from bronchoconstriction and airway edema. Reversibility of airflow limitation may be incomplete in some patients with asthma. For example, airway remodeling can lead to fixed airway narrowing. Structural changes can include thickening of the sub-basement membrane, subepithelial fibrosis, airway smooth muscle hypertrophy and hyperplasia, blood vessel proliferation and dilation, and mucous gland hyperplasia, and hypersecretion.

[0260] Individuals with asthma may experience exacerbations, which are identified as events characterized by a change from the individual's previous status. Severe asthma exacerbations can be defined as events that require urgent action on the part of the individual and his/her physician to prevent a serious outcome, such as hospitalization or death from asthma. For example, a severe asthma exacerbation may require use of systemic corticosteroids (e.g., oral corticosteroids) in a subject whose asthma is usually well controlled without OCS or may require an increase in a stable maintenance dose. Moderate asthma exacerbations can be defined as events that are troublesome to the subject, and that prompt a need for a change in treatment, but that are not severe. These events are clinically identified by being outside the subject's usual range of day-to-day asthma variation.

[0261] Current medications for asthma are typically categorized into two general classes: long-term control medications ("controller medications") such as inhaled corticosteroids (ICS), oral corticosteroids (OCS), long-acting bronchodilators (LABAs), leukotriene modifiers (e.g., leukotriene receptor antagonists or leukotriene synthesis inhibitors, anti-IgE antibodies (omalizumab (Xolair®)), cromolyn and nedocromil, which are used to achieve and maintain control of persistent asthma and quick-relief medications such as short-acting bronchodilators (SABAs), which are used to treat acute symptoms and exacerbations. For purposes of the present invention, these treatments may be referred to as "conventional therapy". Treatment of exacerbations may also include increasing the dose and/or intensity of controller medication therapy. For example, a course of OCS can be used to regain asthma control. Current guidelines mandate daily administration of controller medication or, in many cases, administration of multiple doses of controller medication each day for subjects with persistent asthma (with the exception of Xolair, which is administered every 2 or 4 weeks).

[0262] A subject is generally considered to have persistent asthma if the subject suffers from symptoms on average more than twice a week and/or typically uses a quick relief medication (e.g., SABA) more than twice a week for symptom control. "Asthma severity" can be classified based on the intensity of treatment required to control the subject's asthma once relevant comorbidities have been treated and inhaler technique and adherence have been optimized (see, e.g., GINA Report; Taylor, DR, Eur Respir J 2008; 32:545-554). The description of treatment intensity can be based on the medications and doses recommended in the stepwise treatment algorithm found in guidelines such as NHLBI Guidelines 2007, GINA Report, and their predecessors and/or in standard medical textbooks. For example, asthma can be classified as intermittent, mild, moderate, or severe as indicated in Table X, where "treatment" refers to treatment sufficient to achieve subject's best level of asthma control. (It will be understood that the categories of mild, moderate, and severe asthma in general imply persistent rather than intermittent asthma). One of ordinary skill in the art will appreciate that Table X is exemplary, and that not all of these medications will be available in all healthcare systems, which may affect the assessment of asthma severity in some environments. It will also be appreciated that other emerging or new approaches may affect the classification of mild/moderate asthma. However, the same principle, of mild asthma being defined by the ability to achieve good control using very low-intensity treatment and severe asthma being defined by the requirement for high-intensity treatment, can still be applied. Asthma severity can also or alternately be classified based on intrinsic intensity of the disease in the absence of treatment (see, e.g., NHLBI Guidelines 2007). Assessment can be made on the basis of current spirometry and the patient's recall of symptoms over the previous 2-4 weeks. Parameters of current impairment and future risk may be assessed and included in a determination of the level of asthma severity. Asthma severity may be defined as shown in Figure 3.4(a), 3.4(b), 3.4(c) of the NHLBI Guidelines, for individuals 0-4, 5-11, or ≥ 12 years of age, respectively.

Table X: Treatment-based Asthma Classification

Asthma Classification	Treatment
Intermittent	SABA as needed (typically no more than twice a week)
Mild	Low-dose ICS or other low-intensity treatment (e.g., LTRA, cromolyn, nedocromil, theophylline)
Moderate	Low to moderate dose ICS and LABA or other extra treatment

(continued)

Asthma Classification	Treatment
Severe	High-intensity treatment (high-dose ICS and LABA \pm oral corticosteroids and/or other extra treatment)

[0263] "Asthma control" refers to the extent to which the manifestations of asthma have been reduced or removed by treatment (whether pharmacological or non-pharmacological). Asthma control can be assessed based on factors such as symptom frequency, nighttime symptoms, objective measures of lung function such as spirometry parameters (e.g., %FEV₁ of predicted, FEV₁ variability, requirement for use of SABA for symptom control. Parameters of current impairment and future risk may be assessed and included in a determination of the level of asthma control. Asthma control may be defined as shown in Figure 4.3(a), 4.3(b), or 4.3(c) of NHBLI Guidelines, for individuals 0-4, 5-11, or ≥ 12 years of age, respectively.

[0264] In general, one of ordinary skill in the art can select an appropriate means of determining asthma severity level and/or degree of control, and any classification scheme considered reasonable by those of ordinary skill in the art can be used.

[0265] Provided herein is a compstatin analog of the invention for use in treating persistent asthma in a subject in need thereof, the use comprising administering the complement inhibitor according to an inventive dosing regimen. The subject may suffer from mild or moderate asthma. The subject may suffer from severe asthma. A subject may have asthma that is not well controlled using conventional therapy. A subject may have asthma that, when treated using conventional therapy, requires use of ICS in order to be well controlled. A subject may have asthma that fails to be well controlled despite use of ICS. A subject may have asthma that, if treated using conventional therapy, would require use of OCS in order to be well controlled. A subject may have asthma that fails to be well controlled despite use of high intensity conventional therapy that includes OCS. A long-acting complement inhibitor may be administered as a controller medication or to allow the subject to avoid using or reduce their dose of a conventional controller medication.

[0266] The subject may suffer from allergic asthma, which is the case for most asthmatic individuals. An asthmatic subject may be considered to have allergic asthma if a non-allergic trigger for the asthma (e.g., cold, exercise) is not known and/or is not identified in a standard diagnostic evaluation. An asthmatic subject may be considered to have allergic asthma if the subject (i) reproducibly develops asthma symptoms (or worsening of asthma symptoms) following exposure to an allergen or allergen(s) to which the subject is sensitive; (ii) exhibits IgE specific for an allergen or allergen(s) to which the subject is sensitive; (iii) exhibits a positive skin-prick test to an allergen or allergen(s) to which the subject is sensitive; and/or (iv) exhibits other symptom(s) of characteristic(s) consistent with atopy such as allergic rhinitis, eczema, or elevated total serum IgE. It will be appreciated that a specific allergic trigger may not be identified but may be suspected or inferred if the subject experiences worsening symptoms in particular environments, for example.

[0267] Allergen challenge by inhalation is a technique that is widely used in evaluating allergic airway disease. Inhalation of allergen leads to cross-linking of allergen-specific IgE bound to IgE receptors on, e.g., mast cells and basophils. Activation of secretory pathways ensues, resulting in release of mediators of bronchoconstriction and vascular permeability. Individuals with allergic asthma may develop various manifestations following allergen challenge, e.g., early asthmatic response (EAR), late asthmatic response (LAR), airway hyperreactivity (AHR), and airway eosinophilia, each of which can be detected and quantified as known in the art. For example, airway eosinophilia may be detected as an increase in eosinophils in sputum and/or BAL fluid. The EAR, sometimes referred to as the immediate asthmatic response (IAR), is a response to allergen challenge by inhalation that becomes detectable shortly after the inhalation, typically within 10 minutes (min) of the inhalation, e.g., as a decrease in FEV₁. The EAR typically reaches a maximum within 30 min and resolves within 2-3 hours (h) post-challenge. For example, a subject may be considered to exhibit a "positive" EAR if his/her FEV₁ decreases by at least 15%, e.g., at least 20%, within this time window relative to baseline FEV₁ (where "baseline" in this context refers to conditions before the challenge, e.g., conditions equivalent to the subject's usual condition when not experiencing an asthma exacerbation and not exposed to allergic stimuli to which the subject is sensitive). The late asthmatic response (LAR) typically starts between 3 h and 8 h post-challenge and is characterized by cellular inflammation of the airway, increased bronchovascular permeability, and mucus secretion. It is typically detected as a decrease in FEV₁, which may be greater in magnitude than that associated with the EAR and potentially more clinically important. For example, a subject may be considered to exhibit a "positive" LAR if his/her FEV₁ decreases by at least 15%, e.g., at least 20%, relative to baseline FEV₁ within the relevant time period as compared with baseline FEV₁. A delayed airway response (DAR) may occur beginning between about 26 and 32 h, reaching a maximum between about 32 and 48 h and resolving within about 56 h after the challenge (Pelikan, Z. Ann Allergy Asthma Immunol. 2010, 104(5):394-404).

[0268] The chronic respiratory disorder may be chronic obstructive pulmonary disease (COPD). COPD encompasses a spectrum of conditions characterized by airflow limitation that is not fully reversible even with therapy and is usually

progressive. Symptoms of COPD include dyspnea (breathlessness), decreased exercise tolerance, cough, sputum production, wheezing, and chest tightness. Persons with COPD can experience episodes of acute (e.g., developing over course of less than a week and often over the course of 24 hours or less) worsening of symptoms (termed COPD exacerbations) that can vary in frequency and duration and are associated with significant morbidity. They may be triggered by events such as respiratory infection, exposure to noxious particles, or may have an unknown etiology. Smoking is the most commonly encountered risk factor for COPD, and other inhalational exposures can also contribute to development and progression of the disease. The role of genetic factors in COPD is an area of active research. A small percentage of COPD patients have a hereditary deficiency of alpha-1 antitrypsin, a major circulating inhibitor of serine proteases, and this deficiency can lead to a rapidly progressive form of the disease.

[0269] Characteristic pathophysiologic features of COPD include narrowing of and structural changes in the small airways and destruction of lung parenchyma (in particular around alveoli), most commonly due to chronic inflammation. The chronic airflow limitation observed in COPD typically involves a mixture of these factors, and their relative importance in contributing to airflow limitation and symptoms varies from person to person. The term "emphysema" refers to enlargement of the air spaces (alveoli) distal to the terminal bronchioles, with destruction of their walls. It should be noted that the term "emphysema" is often used clinically to refer to the medical condition associated with such pathological changes. Some individuals with COPD have chronic bronchitis, which is defined in clinical terms as a cough with sputum production on most days for 3 months of a year, for 2 consecutive years. Further information regarding risk factors, epidemiology, pathogenesis, diagnosis, and current management of COPD may be found, e.g., in "Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease" (updated 2009) available on the Global Initiative on Chronic Obstructive Pulmonary Disease, Inc. (GOLD) website (www.goldcopd.org), also referred to herein as the "GOLD Report", the American Thoracic Society/European Respiratory Society Guidelines (2004) available on the ATS website at www.thoracic.org/clinical/copd-guidelines/resources/copddoc.pdf, referred to herein as "ATC/ERS COPD Guidelines" and standard textbooks of internal medicine such as Cecil Textbook of Medicine (20th edition), Harrison's Principles of Internal Medicine (17th edition), and/or standard textbooks focusing on pulmonary medicine.

[0270] The uses disclosed herein may inhibit (interfere with, disrupt) the DC-Th17-B-Ab-C-DC cycle discussed above. For example, administration of a complement inhibitor may break the cycle by which complement stimulates DC cells to promote the Th17 phenotype. As a result, the number and/or activity of Th17 cells diminishes, which in turn reduces the amount of Th17-mediated stimulation of B cells and polyclonal antibody production. These effects may result in "resetting" the immunological microenvironment to a more normal, less pathological state. As described in Example 1, evidence supporting the capacity of complement inhibition to have a prolonged inhibitory effect on Th17-associated cytokine production was obtained in an animal model of asthma.

[0271] Inhibiting the DC-Th17-B-Ab-C-DC cycle may have a disease-modifying effect. Without wishing to be bound by any theory, rather than merely treating symptoms of a disorder, inhibiting the DC-Th17-B-Ab-C-DC cycle may interfere with fundamental pathologic mechanisms that may contribute to ongoing tissue damage even when symptoms are well controlled and/or that may contribute to exacerbations of the disease. Inhibiting the DC-Th17-B-Ab-C-DC cycle may cause a chronic disorder to go into remission. Remission may refer to a state of absence or substantial absence of disease activity in a subject with a chronic disorder, with the possibility of return of disease. Remission may be sustained for a prolonged period of time (e.g., at least 6 months, e.g., 6-12 months, 12-24 months, or more) in the absence of continued therapy or with a reduced dose or increased dosing interval. Inhibition of complement may change the immunological micro-environment of a tissue that is rich in Th17 cells and modify it into a micro-environment that is rich in regulatory T cells (Tregs). Doing so could allow the immune system to "reset" itself and go into a state of remission. For example, remission may be sustained until occurrence of a triggering event. A triggering event may be, for example, an infection (which may result in production of polyclonal antibodies that react both with an infectious agent and a self protein), exposure to particular environmental conditions (e.g., high levels of air pollutants such as ozone or particulate matter or components of smoke such as cigarette smoke, allergens), etc. Genetic factors may play a role. For example, individuals having particular alleles of genes encoding complement components may have a higher baseline level of complement activity, a more reactive complement system and/or a lower baseline level of endogenous complement regulatory protein activity. An individual may have a genotype associated with increased risk of AMD. For example, the subject may have a polymorphism in a gene encoding a complement protein or complement regulatory protein, e.g., CFH, C3, factor B, wherein the polymorphism is associated with an increased risk of AMD.

[0272] An immunologic microenvironment may become progressively more polarized towards a pathological state over time, e.g., in a subject who has not yet developed symptoms of a chronic disorder or in a subject who has developed the disorder and has been treated as described herein. Such a transition may occur stochastically (e.g., due at least in part to apparently random fluctuations in antibody levels and/or affinity) and/or as a result of accumulated "sub-threshold" trigger events that are not of sufficient intensity to trigger a symptomatic outbreak of a disorder.

[0273] It is contemplated that a relatively short course of a long-acting compstatin analog, e.g., between 1 week and 6 weeks, e.g., about 2 - 4 week, may provide a long-lasting benefit. A remission may be achieved for a prolonged period

of time, e.g., 1-3 months, 3-6 months, 6-12 months, 12-24 months, or more. A subject may be monitored and/or treated prophylactically before recurrence of symptoms. For example, a subject may be treated prior to or upon exposure to a triggering event. A subject may be monitored, e.g., for an increase in a biomarker, e.g., a biomarker comprising an indicator of Th17 cells or Th17 cell activity, or complement activation, and may be treated upon increase in the level of such biomarker. See, e.g., PCT/US2012/043845 for further discussion.

IX. Compositions and Administration

[0274] The invention also provides a composition comprising a compstatin analog of the invention.

[0275] Compositions provided herein may comprise a purified cell-reactive, long-acting, or targeted compstatin analog. Purification can be achieved using a variety of approaches that can be selected by one of ordinary skill in the art based to achieve a desired degree of purity with respect to various components present in the composition prior to purification. For example, filtration, high performance liquid chromatography, affinity chromatography, and/or other approaches and combinations thereof can be used. In some embodiments, the composition comprises at least 80%, 85%, 90%, 95%, 98%, 99%, or more cell-reactive, long-acting, or targeted compstatin analog as a percentage of the total compstatin analog by weight. The composition may comprise at least 80%, 85%, 90%, 95%, 98%, 99%, or more cell-reactive, long-acting, or targeted compstatin analog as a percentage of the total compstatin analog on a molar basis. The composition may consist or consist essentially of the compstatin analog.

[0276] A composition comprising a cell-reactive compstatin analog and a compound comprising a cell-reactive functional group may be characterized in that the ratio of the cell-reactive compstatin analog to the compound comprising the cell-reactive functional group on a molar basis is at least 10:1, 20:1, 50:1, 100:1, 500:1, 1,000:1, or more. A composition may comprise at least 80%, 85%, 90%, 95%, 98%, 99%, or more cell-reactive compstatin analog as a percentage of the total compstatin analog by weight. The composition may comprise at least 80%, 85%, 90%, 95%, 98%, 99%, or more cell-reactive compstatin analog as a percentage of the total compstatin analog on a molar basis. A composition may comprise at least 80%, 85%, 90%, 95%, 98%, 99%, or more cell-reactive compstatin analog by weight. A composition may comprise at least 80%, 85%, 90%, 95%, 98%, 99%, or more long-acting compstatin analog by weight. A composition may comprise at least 80%, 85%, 90%, 95%, 98%, 99%, or more targeted compstatin analog by weight. A composition may comprise at least 80%, 85%, 90%, 95%, 98%, 99%, or more targeted compstatin analog by weight. Weight may be dry weight.

[0277] Provided herein is pharmaceutical grade composition comprising a compstatin analog of the invention. The pharmaceutical grade composition can have any of the above-mentioned characteristics in terms of purity in various embodiments. The pharmaceutical grade composition is sufficiently free of endotoxin, heavy metals, and unidentified and/or uncharacterized substances so as to be acceptable, without further purification, as a pharmaceutical composition suitable for administration to a human subject or for the manufacture of a pharmaceutical composition to be administered to a human subject. The pharmaceutical grade composition may be sterile.

[0278] Suitable preparations, e.g., substantially pure preparations of a compstatin analog of the invention, may be combined with pharmaceutically acceptable carriers or vehicles, etc., to produce an appropriate pharmaceutical composition. The term "pharmaceutically acceptable carrier or vehicle" refers to a non-toxic carrier or vehicle that does not destroy the pharmacological activity of the compound with which it is formulated. One of skill in the art will understand that a carrier or vehicle is "non-toxic" if it is compatible with administration to a subject in an amount appropriate to deliver the compound without causing undue toxicity. Pharmaceutically acceptable carriers or vehicles that may be used in the compositions of this invention include, but are not limited to, water, physiological saline, Ringer's solution, sodium acetate or potassium acetate solution, 5% dextrose, and the like. The composition may include other components as appropriate for the formulation desired, e.g., as discussed herein. Supplementary active compounds, e.g., compounds independently useful for treating a subject suffering from a complement-mediated disorder, can also be incorporated into the compositions. The invention provides such pharmaceutical compositions comprising a compstatin analog of the invention and, optionally, a second active agent useful for treating a subject suffering from a complement-mediated disorder.

[0279] Also provided is a pharmaceutically acceptable composition suitable for administration to humans comprising the compstatin analog of the invention, packaged together with a label approved by a government agency responsible for regulating pharmaceutical agents, e.g., the U.S. Food & Drug Administration. Also disclosed is a pharmaceutical kit or pack comprising: (a) a pharmaceutically acceptable cell-reactive, long-acting, or targeted compstatin analog in solid form; (b) a pharmaceutically acceptable carrier or vehicle. Optionally the kit or pack contains instructions for dissolving the cell-reactive, long-acting, or targeted compstatin analog in the carrier. A pharmaceutical kit or pack is also disclosed. The pack or kit comprises sufficient amount of pharmaceutical composition for at least 1 dose, e.g., between 1 and 200 doses or any intervening number or subrange. A pharmaceutical pack or kit may comprise one or more needles and, optionally, one or more syringes. At least one prefilled syringe may be provided. One or more unit dosage forms or premeasured aliquots may be provided. Instructions for administration, which may comprise instructions for self-administration, e.g., via subcutaneous injection, may be provided.

[0280] A pharmaceutical composition can be administered to a subject by any suitable route of administration including, but not limited to, intravenous, intramuscular, subcutaneously, by inhalation, by nasal delivery, intrathecally, intracranially, intraarterially, orally, rectally, transdermally, intradermally, subdermally, etc. A composition comprising a cell-reactive, long-acting, or targeted compstatin analog may be administered intravenously. A composition comprising a cell-reactive, long-acting, or targeted compstatin analog may be administered intra-arterially. The composition can be administered locally, either into the vascular system supplying an organ or tissue, or extra-vascularly in the vicinity of an organ or tissue. It will be understood that "administration" encompasses directly administering a compound or composition to a subject, instructing a third party to administer a compound or composition to a subject, prescribing or suggesting a compound or composition to a subject (e.g., for self-administration), self-administration, and, as appropriate, other means of making a compound or composition available to a subject.

[0281] Pharmaceutical compositions suitable for injectable use (e.g., intravenous administration) or by pump or catheter typically include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. Sterile solutions can be prepared by incorporating the compound in the required amount in an appropriate solvent, optionally with one or a combination of ingredients such as buffers such as acetates, citrates, lactates or phosphates; agents for the adjustment of tonicity such as sodium chloride or dextrose; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid, glutathione, or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and other suitable ingredients etc., as desired, followed by filter-based sterilization. One of skill in the art will be aware of numerous physiologically acceptable compounds that may be included in a pharmaceutical composition. Other useful compounds include, for example, carbohydrates, such as glucose, sucrose, lactose; dextrans; amino acids such as glycine; polyols such as mannitol. These compounds may, for example, serve as bulking agents and/or stabilizers, e.g., in a powder and/or when part of the manufacture or storage process involves lyophilization. Surfactant(s) such as Tween-80, Pluronic-F108/F68, deoxycholic acid, phosphatidylcholine, etc., may be included in a composition, e.g., to increase solubility or to provide microemulsion to deliver hydrophobic drugs. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide, if desired. The parenteral preparation can be enclosed in ampoules, disposable syringes or infusion bags or multiple dose vials made of glass or plastic. Preferably solutions for injection are sterile and acceptably free of endotoxin.

[0282] Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and appropriate other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation can include vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient, e.g., from a previously sterile-filtered solution thereof.

[0283] Oral administration may be used in certain embodiments. Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. A liquid composition can also be administered orally. Formulations for oral delivery may incorporate agents to improve stability within the gastrointestinal tract and/or to enhance absorption.

[0284] For administration by inhalation, a compstatin analog may be delivered in the form of an aerosol spray from a pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide. A metered dose inhaler or nebulizer may be used. The aerosol may comprise liquid particles or dry aerosol (e.g., dry powders, large porous particles, etc.).

[0285] For topical application, a compstatin analog may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutically acceptable compositions can be formulated as a suitable lotion or cream containing a compstatin analog suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol, and water.

[0286] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated may be used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished, e.g., through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are typically formulated into ointments, salves, gels, or creams as generally known in the art.

[0287] The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0288] A compstatin analog or other active compound may be prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. For example, a compstatin analog may be incorporated into or encapsulated in a microparticle or nanoparticle formulation. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, poly-anhydrides, polyglycolic acid, collagen, polyorthoesters, polyethers, polylactic acid, PLGA, etc. Liposomes or other lipid-based particles can be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811 and/or other references listed herein. Depot formulations containing a compstatin analog may be used. The compstatin analog is released from the depot over time, e.g., so as to provide a therapeutic concentration for longer than if the compound was administered intravenously. A CRM may confer depot properties on a compstatin analog of the invention. One of ordinary skill in the art will appreciate that the materials and methods selected for preparation of a controlled release formulation, implant, etc., should be such as to retain activity of the compound.

[0289] It will be appreciated that the compstatin analog and/or additional active agent(s) can be provided as a pharmaceutically acceptable salt. Pharmaceutically acceptable salts include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts, if appropriate depending on the identity of the active agent.

[0290] It will be understood that the pharmaceutically acceptable carriers, compounds, and preparation methods mentioned herein are exemplary and non-limiting. See, e.g., Remington: The Science and Practice of Pharmacy. 21st Edition. Philadelphia, PA. Lippincott Williams & Wilkins, 2005, for additional discussion of pharmaceutically acceptable compounds and methods of preparing pharmaceutical compositions of various types.

[0291] A pharmaceutical composition can be administered in an amount effective to achieve a desired beneficial effect. An effective amount may be sufficient to provide one or more of the following benefits: (i) reduction in at least one symptom or sign of a complement-mediated disorder; (ii) increased quality of life; (iii) reduced hospitalization; (iv) reduced mortality. One of ordinary skill in the art will appreciate that the particular beneficial effect will depend at least in part on various factors, such as the particular disorder being treated. One of ordinary skill in the art will be aware of the symptoms and signs that may occur in subjects with complement-mediated disorders. Examples of symptoms and signs of various complement disorders are described herein. For example, e.g., wherein a subject suffers from PNH or aHUS, a beneficial effect may be a reduction in complement-mediated red blood cell lysis. A beneficial effect may be statistically significant and/or therapeutically meaningful within the judgement of one of ordinary skill in the art.

[0292] A pharmaceutical composition of the invention may be administered parenterally. The composition may be administered intravenously. The composition may be administered by intravenous injection. The composition may be administered as an IV bolus or an IV infusion. The composition may be administered as an IV drip. The composition may be administered as an IV bolus followed by an IV infusion or IV drip. An IV infusion may be administered over about 1, 2, 3, 4, 5, 15, 20, 30, 60, or 120 minutes. An IV drip may be administered over more than about 60 minutes, e.g., over about 1, 2, 3, or more hours. A total amount of between about 0.1 mg/kg/day and about 2,000 mg/kg/day of compstatin analog may be administered, e.g., between about 1 mg/kg/day and about 1,000 mg/kg/day, e.g., between about 5 mg/kg/day and about 500 mg/kg/day. A total amount of between about 10 mg/kg/day and about 100 mg/kg/day of compstatin analog may be administered, e.g., between about 10 mg/kg/day and about 50 mg/kg/day e.g., between about 10 mg/kg/day and about 20 mg/kg/day. Between about 0.5 mg/kg/day to about 10 mg/kg/day of compstatin analog may be administered. Between about 1 mg/kg/day to about 5 mg/kg/day of compstatin analog may be administered. Between about 1 mg/kg/day to about 3 mg/kg/day of compstatin analog may be administered. Between about 3 mg/kg/day to about 5 mg/kg/day of compstatin analog may be administered. Between about 5 mg/kg/day to about 7.5 mg/kg/day of compstatin analog may be administered. Between about 7.5 mg/kg/day to about 10 mg/kg/day of compstatin analog may be administered. It will be appreciated that a variety of different dosing regimens could be used to administer a desired total daily amount. For example, a desired amount of compstatin analog could be administered in a single administration or in multiple administrations, e.g., during a 24 hour period. For example, a subject could receive two or more doses within a 24 hour period, which doses could be administered over the same length of time or over different lengths of time. A cell-reactive, long-acting, or targeted compstatin analog may be administered at time intervals greater than 24 hours. For example, doses could be administered on average every other day, every 3-4 days, weekly, every other week, etc., in various embodiments. Covalently attached, long-acting, or targeted compstatin analogs may protect

cells, tissues, organs, for a period of weeks or months without need for retreatment. For example, subjects may be maintained with retreatment at intervals of between 1-2 weeks, 2-4 weeks, 4-6 weeks, 6-8 weeks, or even longer. Subcutaneous administration may be used to administer at least some doses. For example, administration of approximately 0.1- 5 mg/kg/day, e.g., about 0.5- 2 mg/kg/day is contemplated, e.g., in a volume of about 0.25 ml - 2 mL, e.g., a volume of about 1 ml. The concentration may be about 50 mg/ml to about 300 mg/ml, e.g., about 50 mg/ml - about 100 mg/ml or about 100 mg/ml - about 200 mg/ml. Administration may be daily. Administration may be 1 or 2 times per day. As described further in the Examples, daily subcutaneous administration of an exemplary long-acting compstatin analog readily achieved blood levels well above 5 micromolar. Intramuscular administration may be used to deliver similar amounts of compound. A long-acting compstatin analog may be administered using a therapeutically effective amount to a subject, wherein such administration results in blood concentrations of the compound that achieve a level above at least 1 μ M, at least 2 μ M, at least 2.5 μ M, , at least 3 μ M, at least 4 μ M, at least 5 μ M, at least 6 μ M, at least 7 μ M, at least 8 μ M, at least 9 μ M , at least 10 μ M , at least 11 μ M, at least 12 μ M, or at least 13 μ M, att least 14 μ M, at least 15 μ M, at least 16 μ M, at least 18 μ M, or at least about 20 μ M, or at least about 25 μ M or within any range between 4 μ M and about 15 μ M or about 20 μ M or about 25 μ M. Such level may be maintained for at least about 24 hours, or at least about 48 hours, or at least about 72 hours, or at least about 96 hours, or at least about 120 hours, or at least about 144 hours following a single IV injection or following daily subcutaneous injections for about 5-7 days. Sustained levels may be achieved for longer, e.g., up to about 10 days, 12 days, 14 days, or more. A subject may be treated so as to maintain a steady state level of about 1.0 μ M, about 2.0 μ M, about 2.5 μ M, about 3.0 μ M, about 3.5 μ M, about 4.0 μ M, about 4.5 μ M, about 5.0 μ M, about 5.5 μ M, about 6.0 μ M, about 6.5 μ M, about 7.0 μ M, about 7.5 μ M, about 8.0 μ M, about 8.5 μ M, about 9.0 μ M, about 9.5 μ M, or about 10 μ M. A steady state level may have a value between about 1.0 μ M and about 10.0 μ M, e.g., between about 2.0 μ M and about 5.0 μ M, between about 2.5 μ M and about 5.0 μ M, between about 5.0 μ M and about 7.5 μ M, or between about 7.5 μ M, and about 10 μ M, or any intervening value within any of the afore-mentioned ranges. A concentration may be sufficient to substantially inhibit lysis of red blood cells of PNH patients exposed to human serum in vitro, e.g., using a modified Ham's assay using human serum (see, e.g., Example 8). A concentration may be sufficient to reduce by at least 50%, 60%, 70%, 80%, 90%, or more, lysis of red blood cells of PNH patients exposed to human serum in vitro, e.g., using a modified Ham's assay using human serum (see, e.g., Example 8). In some embodiments a Ham's assay may be performed using human serum adjusted to a magnesium level at about 0.005 mol/L and a pH lowered to about 6.2 to activate complement. Examples 18 and 19 present data confirming the ability of compstatin analogs described herein to inhibit lysis of RBCs from PNH patients.

[0293] Compstatin analogs, e.g., long-acting compstatin analogs, may protect red blood cells of PNH patients from accumulating significant amounts of C3 and/or products of C3 activation on their surface. For example, PNH red blood cells that are protected from complement-mediated lysis by compstatin analogs, e.g., long-acting compstatin analogs, may also be protected from accumulating significant amounts of C3 and/or products of C3 activation on their surface. As known in the art, eculizumab (Soliris®, Alexion Pharmaceuticals, Inc.), is a humanized anti-C5 monoclonal antibody that is approved for treatment of PNH and aHUS in a number of countries (see, e.g., Dmytrijuk A, FDA report: eculizumab (Soliris) for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Oncologist*. 2008 Sep;13(9):993-1000. doi: 10.1634/theoncologist.2008-0086. Epub 2008 Sep 10; Westra D., A new era in the diagnosis and treatment of atypical haemolytic uraemic syndrome. *Neth J Med*. 2012 Apr;70(3): 121-9). It has been reported that when PNH RBCs are exposed to eculizumab they may exhibit accumulation of significant amounts of C3 and/or products of C3 activation on their surface, which may contribute to the clearance and/or extravascular hemolysis of these cells (e.g., in the spleen) and may thus at least in part account for persistent hematologic abnormalities, e.g., persistent anemia, observed in some PNH patients despite treatment with eculizumab. Without wishing to be bound by any theory, this may occur due to inhibition of formation of the MAC by eculizumab, which protects cells from MAC-mediated lysis but does not inhibit C3 activation or deposition of C3 and/or products of C3 activation and leaves PNH cells vulnerable to surface C3 activation and deposition of C3 and/or products of C3 activation due to their lack of GPI-anchored complement inhibiting proteins. Without wishing to be bound by any theory, the ability of compstatin analogs described herein to inhibit C3 activation and thereby inhibit the production of C3 activation products may afford a significant advantage. A compstatin analog of the invention may be used to treat a subject who has been or is being treated with eculizumab and continues to exhibit evidence of hemolysis, e.g., clinically significant hemolysis, such as causing anemia and/or requiring transfusion. A compstatin analog may be used at a concentration sufficient such that the level of C3 and/or C3 activation products on PNH RBCs exposed to the compstatin analog (in vitro (e.g., in a Ham's assay) or in vivo) is within the range exhibited by normal RBCs from healthy subjects. The level of C3 and/or C3 activation products on PNH RBCs exposed to the compstatin analog (in vitro (e.g., in a Ham's assay) or in vivo) may be within about 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, or 5.0 of the average level or upper limit of normal. The level of C3 and/or C3 activation products on PNH RBCs exposed to a compstatin analog (in vitro (e.g., in a Ham's assay) or in vivo) may be less than the level of of C3 and/or C3 activation products on PNH RBCs exposed to Soliris at a concentration that provides equivalent protection against complement-mediated lysis. The level of C3 and/or C3 activation products on PNH RBCs exposed to a compstatin analog (in vitro

or in vivo) may be no more than about 5%, 10%, 15%, 20%, 25%, 30%, 40%, or 50% of the level of C3 and/or C3 activation products on PNH RBCs exposed to eculizumab at a concentration that provides equivalent protection against complement-mediated lysis. The level of C3 and/or C3 activation products on PNH RBCs exposed to the compstatin analog (in vitro or in vivo) may be within about 1.2, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, or 5.0 of the average level or upper limit of a normal range. The PNH cells may comprise or consist of Type II PNH cells, Type III PNH cells, or a mixture thereof. The RBCs may be at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or more Type III and/or Type II RBCs. The cells may comprise some Type I cells. RBCs may be classified as Type I, II, or III based on the level of a GPI-anchored protein such as CD59 on their surface, which may be measured using flow cytometry, immunofluorescence, or ELISA, e.g., using an antibody (e.g., a monoclonal antibody) or other binding agent that binds to said GPI-anchored protein. Inhibition of deposition of C3 and/or products of C3 activation on cells or surfaces may be used as an indicator of efficacy of a compstatin analog in other complement-mediated diseases such as aHUS, other complement-mediated hemolytic diseases, or other complement-mediated diseases. For example, a compstatin analog may inhibit such deposition on endothelial cells in a subject with aHUS. The level of C3 and/or C3 activation product(s) may be measured using flow cytometry, immunofluorescence, or ELISA, e.g., using an antibody (e.g., a monoclonal antibody) or other binding agent that binds to C3 and/or to one or more C3 activation product(s). A C3 activation product may be C3b, C3c, or C3d. A binding agent may bind to C3d. A binding agent may bind to C3d and at least one other C3 activation product. PNH patient RBCs contacted with a compstatin analog in vitro (e.g., in a Ham's assay) may be protected from activated complement such that the relative proportion (percentages) of Type I, Type II, and Type III cells or the relative proportion or percentages of Type III and Type I, Type II and Type I, or Type III and Type II, are approximately the same as in a control assay in which inactivated complement (e.g., heat inactivated complement) is used. PNH patient RBCs contacted with a compstatin analog in vitro (e.g., in a Ham's assay) may be protected from activated complement such that the relative proportion or percentages of Type I, Type II, and Type III cells or the relative proportion or percentages of Type III and Type I, Type II and Type I, or Type III and Type II, are within 5% of the proportions or percentages obtained in a control assay in which inactivated complement (e.g., heat inactivated complement) is used. Complement may be inactivated by heat inactivation, which may be performed by heating complement components or serum or plasma containing complement components to 56 degrees C or higher.

[0294] Measurements of LDH (an enzyme that is abundant in red blood cells and can function as a marker for hemolysis), one or more hematologic parameters such as hematocrit, hemoglobin, and/or reticulocyte measurements may additionally or alternately be used in determining the amount of lysis. One or more such methods may be used to determine the amount of lysis of RBCs, e.g., RBCs that are susceptible to complement-mediated lysis, e.g., PNH patient cells, aHUS patient cells, cells from subjects with other complement-mediated hematologic disorders, cells exposed to abnormally high levels of complement activation. A compstatin analog may be used in a method comprising contacting one or more cells in vitro or in vivo with the compstatin analog and measuring the effect of the compstatin analog on one or more indicators of complement-mediated cell damage and/or cell surface complement activation or deposition. Contacting the one or more cells for a sufficient time at a sufficient concentration may result in a reduction of an abnormally high value or an increase in an abnormally low value to within a normal range or to within 5%, 10%, 15%, 20%, or 25% of the lower or upper limit of a normal range.

[0295] A compstatin analog may be used in treating a complement-mediated hemolytic disease such as PNH, wherein such use comprises selecting or modifying a dosing regimen or one or more components of a dosing regimen for a patient in need thereof. The one or more components of a dosing regimen may comprise a dose, dosing interval, route of administration (e.g., IV or subcutaneous), or combination thereof. A dose may be a loading dose, maintenance dose, or both. One or more blood samples may be obtained from a patient and a dosing regimen or component thereof for a compstatin analog, e.g., a long-acting compstatin analog, may be selected or modified to achieve a desired level of protection of the patient's RBCs from lysis and/or from accumulation of C3 and/or C3 activation product(s) in vitro. One or more doses of a compstatin analog, e.g., a long-acting compstatin analog, may be administered to a patient, and one or more blood samples may be subsequently obtained from a patient and assessed for level of C3 and/or C3 activation product(s) on their surface. A dosing regimen or component thereof, e.g., a dose, dosing interval, or route of administration, may be selected or modified to achieve a desired level of protection of the patient's RBCs from lysis and/or from accumulation of C3 and/or C3 activation products in vitro or in vivo. A dosing regimen or component thereof, e.g., dose, dosing interval, or route of administration, may be selected or modified to achieve a desired level of protection of the patient's RBCs from extravascular clearance and/or extravascular lysis in vivo. A desired level may be, e.g., a level that is accepted in the art as providing a clinically meaningful benefit, a level that provides a clinically meaningful benefit to a particular patient, a level that is within the normal range, a level selected by a medical practitioner, or any other selected level. A normal range for a parameter may be known in the art and/or may be a reference range established by a laboratory, e.g., a clinical laboratory, wherein the value of the relevant parameter as measured in at least 95%, 96%, 97%, 98%, or 99% of the general population or at least 95%, 96%, 97%, 98%, or 99% of healthy individuals (which may optionally be matched for one or more demographic variables such as gender, age, etc.) or biological specimens obtained therefrom (such as blood samples) would fall within the reference range. A reference range may be established using

a sample population representative of the general population or representative of healthy individuals.

[0296] A long-acting compstatin analog comprising a CRM may be designed to confer a slower rate of systemic absorption after subcutaneous or intramuscular administration to a subject as compared with a compstatin analog not comprising a CRM. Particular CRM properties, e.g., length, may be selected to confer a desired rate of systemic absorption after subcutaneous or intramuscular administration as compared with at least some other CRMs. The C_{max} may be reduced in comparison to a comparable dose of a compstatin analog not linked to a CRM, which may thereby contribute to keeping the plasma concentration within a desired window, e.g., the therapeutic window, for the compound. A long-acting compstatin analog composition may be characterized in that a dose, when administered subcutaneously, appears fully absorbed within about 1, 2, 3, 4, 6, 8, 12, 15, 30, 45, 60, 90, or 120 hours following administration based on visual observation at the injection site.

[0297] It will be understood that there may be an initial treatment phase during which treatment is more frequent and/or in which higher doses are administered. For example, in a subject with PNH or aHUS, it may require several doses to achieve protection of a substantial fraction of the subject's RBCs. After that, lower doses and/or less frequent dosing could be used, e.g., to protect newly formed RBCs and/or to replenish protection of existing RBCs. Of course similar approaches may be followed for treatment of any disease where appropriate. Treatment may be started using IV administration and then switched to subcutaneous, intramuscular, or intradermal for maintenance therapy. Depending on the disease, treatment may continue at intervals for, e.g., months, years, or indefinitely. Appropriate doses and dosing regimen depend at least in part upon the potency and half-life of the compstatin analog (or other active agent), and may optionally be tailored to the particular recipient, for example, through administration of increasing doses until a preselected desired response is achieved, such as a desired degree of complement inhibition and/or cell protection. If desired, the specific dose level for any particular subject may be selected based at least in part upon a variety of factors including the activity of the specific compound employed, the particular condition being treated, the age, body weight, general health, route of administration, the rate of excretion, any drug combination, and/or the degree of complement protein expression or activity measured in one or more samples obtained from the subject.

[0298] The invention encompasses a compstatin analog of for use in combination with additional therapy. Such additional therapy may include administration of any agent(s) used in the art or potentially useful for treating a subject suffering from the disease.

[0299] When two or more therapies (e.g., compounds or compositions) are used or administered "in combination" with each other, they may be given at the same time, within overlapping time periods, or sequentially (e.g., separated by up to 2 weeks in time). They may be administered via the same route or different routes. The compounds or compositions may be administered within 48 hours of each other. A compstatin analog can be given prior to or after administration of the additional compound(s), e.g., sufficiently close in time that the compstatin analog and additional compound(s) are present at useful levels within the body at least once. The compounds or compositions may be administered sufficiently close together in time such that no more than 90% of the earlier administered composition has been metabolized to inactive metabolites or eliminated, e.g., excreted, from the body, at the time the second compound or composition is administered.

[0300] Examples falling outside the scope of the appended claims are provided for reference.

Example 1: Development of PEGylated Compstatin Analogs that Retain Substantial Complement Inhibiting Activity

[0301] A compstatin analog having the amino acid sequence of the compstatin analog of SEQ ID NO: 28, but incorporating an AEEAc-Lys moiety located C-terminal to the Thr residue of SEQ ID NO: 28 for purposes of subsequent conjugation of an NHS ester activated PEG to the amino group of the Lys side chain was synthesized. The compound was synthesized using standard methods. Briefly, amino acids (including AEEAc) were obtained as Fmoc-protected amino acids, in which the α -amino group of each amino acid was protected with Fmoc. Side chain functional groups were also blocked with various appropriate protective groups. Synthesis was accomplished following the solid phase methodology described by Merrifield (J. Amer. Chem. Soc. 85, 2149 (1963)). Chain assembly was performed on solid phase, at the conclusion of which the N-terminus was acetylated; the peptide was then cleaved from the solid phase and simultaneously deprotected via acidolysis using TFA and amidated. The linear peptide was then oxidized and purified. The resulting compstatin analog is represented as follows Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-AEEAc-Lys-NH₂ (SEQ ID NO: 51), abbreviated as CA28-AEEAc-Lys. Note that for purposes of brevity, the N-terminal acetyl group and C-terminal amino groups are omitted in this abbreviation. Monofunctional, linear NHS-ester activated PEGs with molecular weights of 30 kD and 40 kD (NOF America Corp. White Plains, NY, Cat. No. SUNBRIGHT® ME-300GS and Cat. No. SUNBRIGHT® ME-400GS), respectively, were coupled to the lysine side chain of CA28-AEEAc-Lys, resulting in long-acting compstatin analogs represented as follows: CA28-AEEAc-Lys-(PEG30k) and CA28-AEEAc-Lys-(PEG40k), and purified. Note that the number after the term "PEG" and preceding the letter "k" represents the molecular weight of the PEG moiety in kilodaltons, and the "k" is an abbreviation for kD). CA28-AEEAc-

Lys-(PEG30k) is also referred to as CA28-1. CA28-AEEAc-Lys-(PEG40k) is also referred to as CA28-2.

[0302] Inhibitory activity of the synthesized compounds was assessed by measuring the effect of the compounds on complement activation via the classical pathway using a standard complement inhibition assay. The protocol measures C3b deposition in an ELISA format. C3b deposition monitored using this method is generated through complement activated by the classical pathway. Briefly, 96-well plates are coated with BSA. Human plasma, chicken ovalbumin (OVA), polyclonal anti-OVA antibodies and compound being tested (referred to as "drug") are added and incubated, followed by addition of Anti-human C3 HRP-conjugated antibody. After an additional incubation, substrate is added and signal detected. Details of the protocol are as follows:

[0303] Protocol for Classical Complement Inhibition Assay

Materials:

[0304]

- Ninety-six well plate (polystyrene plate, Thermo Scientific, 9205)
- Chicken OVA (Sigma A5503-5G)
- Rabbit anti-chicken OVA (Abcam ab1221)
- Blocking buffer (Startingblock buffer, Thermo Scientific 37538)
- Veronal Buffer (5X concentration, Lonza 12-624E)
- Human plasma (collected with Lepirudin at 50 ug/ml final concentration)
- Goat anti-human C3 HRP-conjugated Ab (MP Biomedicals, 55237)
- Tween-20 Wash Buffer (0.05% Tween 20-PBS buffer)
- TMB (Peroxidase substrate, BD 555214) - 1:1 mixture of BD 51-2607KC and 51-2606KC.
- 1M H₂SO₄

Protocol:

[0305]

1. Add 100 ul/well of 1% chicken OVA (in PBS)
2. Incubate overnight @ 4°C or room temperature for 1-2 hr.
3. Remove by shaking and tapping the plate.
4. Block by adding 200ul of blocking buffer
5. Incubate for 1 h at room temp
6. Remove by shaking and tapping the plate
7. Add 100 ul of 1:1000 dilution of Polyclonal anti-chicken OVA in blocking buffer
8. Incubate for 1h at room temp
9. Wash twice with wash buffer
10. Add 50 ul VB⁺⁺ to wells #2 to 12
11. Add 100ul of starting drug dilution (2X in VB⁺⁺) to well 1.
12. Serially dilute (1:2) the drug from wells 1 to 10 as follow
 - a. Take 50ul of solution from the originating well
 - b. Add this to the next well
 - c. Mix by pipetting several times
 - d. Repeat up to well #10

Note: from well #10 remove 50ul and discard.

13. Add 50ul of 2X plasma (1:37.5 dilution of original plasma) dilution to wells 1 to 11
14. Incubate for 1h
15. Wash with wash buffer
16. Add 100ul of 1/1000 dilution of anti-C3-HRP Ab in blocking buffer
17. Incubate for 1h
18. Wash with wash buffer
19. Add 100ul of TMB to all wells
20. Incubate for 5-10 min in dark
21. Add 50 ul 1M H₂SO₄
22. Read the plate at 450nm

VB⁺⁺**Formula:****[0306]**

Barbital	5mM
NaCl	72.5 mM
MgCl ₂	0.5 mM
CaCl ₂	0.15 m M
PH	7.4

Stock solutions:**Veronal Buffer (5X)****[0307]**

	Prod #	MW	For 500ml
9 mM Sodium Barbitone	Sigma B0500	206.17	927 mg
15.5 mM diethylbarbituric acid	Sigma B0375	184.19	1.42 grams

Mg-C12 (200X)**[0308]**

	Prod #	MW	For 50 ml
100 mM MgCl ₂ -6H ₂ O	Sigma M0250	203.30	1.00 gram

CaCl₂ (500x)**[0309]**

	Prod #	MW	For 50 ml
75mM CaCl ₂	Sigma C7902	147.01	551.28 mg

[0310] To prepare 50 ml of working buffer:

- Weight 210 mg NaCl
- Add 10ml of 5X VB
- Add 100ul of CaCl₂ (500X)
- Add 250ul MgCl (200X)
- Adjust volume to 50 ml with H₂O
- Adjust pH to 7.4

[0311] Data was analyzed using GraphPad Prism5 software. Data sets from each experiment were normalized to percent activation compared to the 100% activation control corresponding to the well to which no compound is added. Drug concentration values (X values) were transformed to their logarithms, and percent activation (Pa) (Y values) was

transformed to percent inhibition (Pi) using the following formula $Pi=100-Pa$ ($Yi=100-Ya$). The percent inhibition was plotted against the drug concentration and the resulting data set was fit to a sigmoidal-dose response function [$Y=Bottom+(Top-Bottom)/(1+10((\log EC-X)))$]. IC_{50} values were obtained from the fit parameters.

[0312] Results are presented in Figure 1, and the IC_{50} values are shown in Table 2 (in Example 2). As indicated, CA28-1 and CA28-2 displayed about 30% of the activity of CA28 on a molar basis.

Example 2: Development of Long-Acting Compstatin Analogs that Demonstrate Increased Molar Activity

[0313] Eight-arm NHS-ester activated PEG with molecular weight of 40 kD (NOF America Corp. White Plains, NY, Cat. No. SUNBRIGHT® HGEO-400GS; chemical formula: hexaglycerol octa(succinimidylxyglutaryl) polyoxyethylene) was coupled to the lysine side chain of CA28-AEEAc-Lys, resulting in long-acting compstatin analogs represented as follows: (CA28-AEEAc)₈-PEG40k, also referred to as CA28-3. Complement inhibiting activity of CA28-3 was tested using the assay described in Example 1. Results are plotted in Figure 1 and IC_{50} value is listed in Table 2, both as a function of CA28 concentration. The concentration of CA28 was calculated using the extinction coefficient of CA28 at 283 nm (10208.14 L·mol⁻¹·cm⁻¹). Based on other analysis (UV absorption vs. mass of material, and elemental CHN% analysis) it was concluded that there are 7.5 CA28 moieties per molecule of CA28-3. Thus, the activity of CA28-3 on a molar basis is 7.5-fold higher than shown in Figure 1 and Table 2. Thus, the IC_{50} value in Table 2 is 7.5-fold higher than the actual IC_{50} of CA28-3 on a molar basis. The IC_{50} of CA28-3 on a molar basis is calculated as about 0.26 (lower than that of the parent compound CA28). Figure 2 shows percent complement activation inhibiting activity of CA28 and long-acting compstatin analogs CA28-2 and CA28-3, as a function of CA28-3 concentration (μM), i.e., the activity of CA28-3 has been corrected to account for the fact that the compound contains 7.5 CA28 moieties. On a molar basis, the complement inhibiting activity of CA28-3 exceeds that of CA28.

Table 2

	CA28	CA28-1	CA28-2	CA28-3
IC_{50}	0.3909	1.264	1.288	1.927

[0314] The solubility of CA28-1, CA28-2, and CA28-3 in water with or without a variety of buffer substances and/or excipients was observed to exceed that of the parent compound CA28.

Example 3: Long-Acting Compstatin Analogs that Demonstrate Dramatically Increased Plasma Half-Life and C_{max}

[0315] This Example describes determination of pharmacokinetic parameters of long-acting compstatin analogs CA28-2 and CA28-3 following administration to Cynomolgus monkeys.

Dosing and Sample Collection

[0316] CA28-2 and CA28-3 were administered at time 0 via intravenous injection into female Cynomolgus monkeys (three per group, 2-5 yrs old, 2.9-3.5 kg). Compounds were administered at 50 mg/kg in 5% dextrose in water at a concentration of 25 mg/ml. Blood specimens (~1 mL each) were collected from the femoral vein at the following timepoints: Pre-dose, 5 min, 15 min, 30 min, 1 hour (h), 4 h, 8 h, 24 h, 48 h, 96 h (4days), and 192 h (8 days) post dose. Specimens were collected via direct venipuncture and placed into a red top serum tube containing no anticoagulant, and kept at room temperature for at least 30 minutes. Blood samples were centrifuged at a temperature of 4°C at 3000 x g for 5 minutes. Samples were maintained chilled throughout processing. Serum samples were collected after centrifugation and placed into sample tubes. Samples were stored in a freezer set to maintain -60°C to -80°C. All animals showed normal activity throughout the study. No compound-related abnormalities were noted in the animals throughout the study.

[0317] Sample Analysis. Plasma samples obtained as described above were analyzed by LC/MS/MS using the following methods to determine the concentration of compound: 50 μL of sample was mixed with internal standard (CA28-AEEAc-Arg) and then 100 μL of 1 M NH₄OAc, pH 3.5 with HOAc was added and mixed. Then 250 μL of acetonitrile was added and mixed. The sample was centrifuged and supernatant poured into another tube and dried. The sample was reconstituted and injected onto the LC/MS/MS system. Mobile phase A was 5 mM NH₄OAc with 0.1% FA and Mobile Phase B was 90:10 (ACN:50 mM NH₄OAc) with 0.1% FA. The LC column was the Intrada WP-RP 2x150 mm, 3 μm. Quantitation was on an Applied Biosystems API-4000 triple quadrupole mass spectrometer operated in positive ion mode. In-source collision induced dissociation (CID) was used to fragment the compound in the mass spectrometer source and the m/z 144 ion was mass selected in Q1, fragmented, and the m/z 77 ion mass selected in Q3 and detected.

Data was processed using Analyst 1.4.2 software.

[0318] Results. The serum concentrations in micrograms/ml of CA28-2 and CA28-3 at each time point are presented in Table 3 below. Data for each of 3 monkeys that received the indicated compound are shown. Average values and standard deviations are readily calculated. There was notable consistency between animals. CA28 are historical data obtained in a previous study in which CA28 was administered intravenously to Cynomolgus monkeys. In that study, CA28 was detected in samples using HPLC.

Table 3
Serum Concentration in ug/mL

Time (days)	CA28-3(8-arm 40k PEG) (50 mg/kg)			CA28 (200 mg/kg)	CA28-2 (linear 40k PEG) (50 mg/kg)		
	mg/kg						
0.0035	1600	1330	1300		1460	1660	1610
0.01	1600	1220	1480		1360	1430	1530
0.02	1510	1170	1270	34	1310	1510	1500
0.042	1270	1030	1220	17	1290	1340	1540
0.167	926	893	934	9	1200	1210	1390
0.333	797	714	792	5		1190	1180
1	621	479	558	1.5	927	853	881
2	384	355	360		612	733	760
4	280	252	262		461	458	424
8	151	136	136		268	282	293

[0319] Results for each compound were averaged and are plotted in Figure 3. A remarkable increase in half-life and C_{max} was observed for both CA28-2 and CA28-3 compared to CA28. The terminal half-lives of both CA28-2 and CA28-3 were around 4 - 4.5 days. Based on these data, it is expected that intravenous administration at approximately 1-2 week dosing intervals will provide sustained levels of compound and effectively inhibit complement activation in human subjects, though shorter or longer dosing intervals may be used.

Example 4: Long-Acting Compstatin Analog Comprising HSA as a Clearance Reducing Moiety

[0320] Side chain lysines of human serum albumin (HSA) were converted to thiols using 2-iminothiolane and reacted with a compstatin analog comprising a maleimide as a reactive functional group: Ac-Ile-Cys*-Val-(1-Me)-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-AEEAc-Lys-(C(=O)-(CH₂)₅-Mal)-NH₂ (SEQ ID NO: 68). The resulting long-acting compstatin analog (CA28-4) was tested in vitro for complement inhibiting activity (Figure 4) as described in Example 1 and in vivo for pharmacokinetic properties as described in Example 3. Pharmacokinetic parameters of CA28-4 following administration to Cynomolgus monkeys were determined as described in preceding example. Results are shown in Figure 5 (along with results for CA28, CA28-1, CA28-2, and CA28-3). PK data for CA28-4 are presented in Table 4.

Table 4
Serum Concentration in ug/mL

Time (hr)			
0.0035	1790.0	1445.00	1395.00
0.0100	1195.0	915.50	835.00
0.0200	900.0	504.50	553.50
0.0420	4490	267.50	295.00
0.1670	194.0	164.00	158.50
0.3330	150.0	163.00	119.50
1.0000	97.2	86.00	78.05
2.0000	73.3	51.55	57.40
4.0000	43.1	29.20	34.15
8.0000	24.1	16.25	20.00

Example 5: Synthesis and Activity of PEG-Based Compstatin Analogs Using Different NHS-Activated PEGs

[0321] A compstatin analog having the amino acid sequence of the compstatin analog of SEQ ID NO: 28, but incorporating an AEEAc-Lys moiety located C-terminal to the Thr residue of SEQ ID NO: 28 for purposes of subsequent conjugation of an NHS ester activated PEG to the amino group of the Lys side chain was synthesized as described in Example 1. The resulting compstatin analog is represented as follows Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-AEEAc-Lys-NH₂ (SEQ ID NO: 51), abbreviated as CA28-AEEAc-Lys. Monofunctional, linear monomethoxy-NHS-activated ester/carbonate PEGs with molecular weights of 40 kD and differing in terms of the NHS carboxylate attachment chemistry (NOF America Corp. White Plains, NY, Cat. Nos. SUNBRIGHT® ME-400CS, SUNBRIGHT® ME-400GS, SUNBRIGHT® ME-400HS, SUNBRIGHT® ME-400TS) were coupled to the lysine side chain of CA28-AEEAc-Lys via an amide bond. (The Lys residue is Lys15 since the AEEAc linker contains an amino acid residue.) All compounds were acetylated on the N-terminus, amidated on the C-terminus, and cyclized via a disulfide bond between Cys2 and Cys12. (The acetylation, amidation, and cyclization were performed prior to coupling to the PEG.) The compounds were prepared as trifluoroacetate salts and were purified. The compounds are represented as shown in the following table (Table 5). The letters CS, GS, HS, and TS represent the different linker moieties between the PEG moiety and the NHS moiety as indicated in further detail in Table 5. It will be understood that various names and abbreviations for each compound may be used interchangeably. Note that CA28-2 (see Example 1) is the same as CA28-2GS.

Table 5: PEG-Based Compstatin Analogs Containing One Compstatin Analog Moiety

Compound Abbreviation and ID	Compound Name*,†	Activated PEG
CA28-2CS	CA28-AEEAc-LysCS = CA28-AEEAc-Lys(mPEG40K-succinyl)	PEG40K: Methoxy-PEG-CO(CH ₂) ₂ COO-NHS (NOF Sunbright 400CS) Chemical Name: α-Succinimidylsuccinyl-ω-methoxy, polyoxyethylene CAS#: 78274-32-5
CA28-2GS (also referred to as CA28-2)	CA28-AEEAc-LysGS CA28-AEEAc-Lys(mPEG40K-pentanedioyl)	PEG40K: Methoxy-PEG-CO(CH ₂) ₃ COO-NHS (NOF Sunbright 400GS) Chemical Name: α-Succinimidylglutaryl-ω-methoxy, polyoxyethylene CAS#: 111575-54-3
CA28-2HS	CA28-AEEAc-LysHS CA28-AEEAc-Lys(mPEG40K-hexanoyl)	PEG40K: Methoxy-PEG-(CH ₂) ₅ COO-NHS (NOF Sunbright 400HS) Chemical Name: Poly(oxy-1,2-ethanediyl), α-methyl-ω-{2-[(2,5-dioxo-1-pyrrolidinyl)oxy]-6-oxohexyloxy}-
CA28-2TS	CA28-AEEAc-LysTS CA28-AEEAc-Lys(mPEG40K-carbonyl)	PEG40K: Methoxy-PEG-COO-NHS (NOF Sunbright 400TS) Chemical Name: α-Succinimidyl carbonyl-ω-methoxy, polyoxyethylene CAS# 135649-01-3
*AEEAc=8-Amino-3,6-dioxa-octanoyl †Compounds were prepared as trifluoroacetate salts but other counterions could be used		

[0322] Compounds were analyzed by reverse phase HPLC. Figure 6 shows a representative chromatogram for one of the compounds. A VariTide RPC column was used. Eluent A was 0.1% TFA in water; Eluent B was 0.1% TFA in 50% CAN/40% water. Flow rate was 1.000 ml/min with a gradient of 0%B to 100%B over 40 minutes.. The peak with a retention time of 33.68 minutes represents the PEGylated compound and has a relative area of 96.50%.

[0323] Inhibitory activity of the compounds was assessed by measuring the effect of the compounds on complement activation via the classical pathway using a standard complement inhibition assay as described in Example 1. Results are plotted in Figure 7. These results represent a combination of two separate experiments. The compounds showed notably similar complement inhibiting activity.

Example 6: Synthesis and Activity of Bifunctionalized PEG-Based Compstatin Analogs

[0324] Bifunctional, linear monomethoxy-NHS-activated ester/carbonate PEGs with molecular weights of 40 kD and differing in terms of the NHS carboxylate attachment chemistry were obtained from NOF America Corp. (White Plains, NY). The activated PEGs were coupled to the lysine side chain of CA28-AEEAc-Lys via an amide bond such that two CA28-AEEAc-Lys moieties were coupled to each PEG chain. All compounds were acetylated on the N-terminus and amidated on the C-terminus of the CA28-AEEAc-Lys moieties, and cyclized via a disulfide bond between Cys2 and Cys12. (The acetylation, amidation, and cyclization were performed prior to coupling to the PEG.) The compounds were prepared as acetate salts and were purified. The compounds are represented as shown in the following table (Table 6).

Table 6: Bifunctionalized PEG-Based Compstatin Analogs

Compound Abbreviation and ID	Compound Name*,†	Activated PEG
CA28-2CS-BF	CA28-AEEAc-LysCS = CA28-AEEAc-Lys(mPEG40K-succinyl)	PEG40K: NHS-OCO(CH ₂) ₂ COO-PEG-CO(CH ₂) ₂ COO-NHS Chemical Name: α -Succinimidyloxysuccinyl- ω -succinimidyloxysuccinyloxy, polyoxyethylene CAS#: 85419-94-9
CA28-2GS-BF	CA28-AEEAc-LysGS CA28-AEEAc-Lys(mPEG40K-pentanedioyl)	PEG40K: NHS-OCO(CH ₂) ₃ COO-PEG-CO(CH ₂) ₃ COO-NHS Chemical Name: α -Succinimidyloxyglutaryl- ω -succinimidyloxyglutaryloxy-, polyoxyethylene CAS#: 154467-38-6
CA28-2HS-BF	CA28-AEEAc-LysHS CA28-AEEAc-Lys(mPEG40K-hexanoyl)	PEG40K: NHS-OCO(CH ₂) ₅ O-PEG-(CH ₂) ₅ COO-NHS Chemical Name: α -[6-[(2,5-dioxo-1-pyrrolidinyl)oxy]-6-oxohexyl]- ω -[6-[(2,5-dioxo-1-pyrrolidinyl)oxy]-6-oxohexyloxy]-, polyoxyethylene
CA28-2TS-BF	CA28-AEEAc-LysTS CA28-AEEAc-Lys(mPEG40K-carbonyl)	PEG40K: NHS-OCO-PEG-COO-NHS Chemical Name: α -Succinimidyl carbonyl-co-Succinimidyl carbonyl, polyoxyethylene
*AEEAc=8=Amino-3,6-dioxa-octanoyl †Compounds were prepared as acetate salts but other counterions could be used		

[0325] Inhibitory activity of CA28-2GS-BF was assessed by measuring the effect of the compound on complement activation via the classical pathway using a standard complement inhibition assay as described in Example 1 and analyzed as described in Example 1. Results are plotted in Figure 8. As described above, CA28-2GS-BF contains two compstatin analog moieties per molecule. Although the activity per compstatin analog moiety of CA28-2GS-BF is less than the activity of an individual CA28 molecule, the activity of the two compounds on a molar basis is virtually identical over a broad range of concentrations.

Example 7: Subcutaneous Administration of Bifunctionalized PEG-Based Compstatin Analog

[0326] This Example describes determination of pharmacokinetic parameters of long-acting compstatin analog CA28-2GS-BF following administration to Cynomolgus monkeys either via a single intravenous (IV) injection or with repeated (once daily) subcutaneous administration for seven days.

[0327] Dosing and Sample Collection CA28-2GS was administered at time 0 via intravenous injection or via repeat subcutaneous injection (daily, for seven days) into male Cynomolgus monkeys. Six non-naive male Cynomolgus monkeys, age 1-5 years, ranging in weight from 4.6 to 5.3 kilograms, were used in this study (three per group). The animals were healthy at the start of the trial. seven days. The study was not blinded. Animals were supplied with water ad libitum and a commercial diet twice daily prior to initiation of the study. Food was supplied to the animals per facility SOP prior to the study. Animals were not fasted. Animals were dosed via intravenous and subcutaneous administration at time 0 on the appropriate day. A size 22 gauge needle was used for the subcutaneous administration. The compound was administered at 50 mg/kg in 5% dextrose in water at a concentration of 25 mg/ml. Blood specimens (~1 mL each) were collected from the femoral vein at the following timepoints: Day 1: Pre-dose, 5 min, 15 min, 30 min, 1 hour (h), 4 h, 8 h. Days 2 - 9: 0 min. Day 16: Final sample based on Day 1 dosing. Each blood sample (~1.0 mL) was collected from the

monkey's femoral or saphenous vein via direct venipuncture and placed into a red top serum tube containing no anti-coagulant, and kept at room temperature for at least 30 minutes. Blood samples were centrifuged at a temperature of 4°C at 3000 xg for 5 minutes. Samples were maintained chilled throughout processing. Serum samples were collected after centrifugation and placed into sample tubes. Samples were stored in a freezer set to maintain -60°C to -80°C.

Serum samples and leftover dosing solutions were shipped frozen on dry ice for analysis.

[0328] The site of each subcutaneous administration was observed to see how fast the injection volume was absorbed and also to see if the formulation left behind a lump or fully went away. The dose sites were observed at each collection timepoint and in the afternoon of days 2-7. All doses were absorbed during the duration of the study. Based on the observations it is estimated that doses were absorbed within fifteen minutes after administration. All animals showed normal activity throughout the study. No compound-related abnormalities were noted in the animals throughout the study.

[0329] **Sample Analysis.** Plasma samples obtained as described above were analyzed by LC/MS/MS leveraging CID (collision induced degradation) similarly to the method described in Example 3.

[0330] **Results.** Serum concentration vs time for CA28-2GS-BF when administered IV or subcutaneously as described above are plotted in Figure 9. The data points represent all PEGylated CA28 compound detected. CA28 data shown on Figure 9 are historical data obtained in a previous study in which CA28 was administered intravenously to Cynomolgus monkeys. In that study, CA28 was detected in samples using HPLC.

[0331] A peak serum concentration of 500 µg/ml (11 µM) was achieved by subcutaneous administration of CA28-2GS-BF. The terminal half-life of CA28-2GS-BF was approximately 5 days when administered either IV or subcutaneously. Results are summarized in the tables below:

Table 7

Summary of Study Sample Concentrations for CA28-2GS-BF in Monkey Serum (IV @ 50 mg/kg on Day 0)			
	CA28-2GS-BF Cone. (µg/mL)		
Timepoint	Animal 1	Animal 2	Animal 3
5 min	1850	1550	2030
15 min	1760	1440	2000
30 min	1560	1380	1810
1 hr	1650	1330	1710
4 hr	1270	1000	1510
8 hr	1050	913	1360
Day 2	684	661	711
Day 3	541	471	538
Day 4	463	417	492
Day 5	366	384	389
Day 6	346	331	358
Day 7	303	306	311
Day 8	257	252	259
Day 9	217	252	233
Day 15	92.8	107	95.6

Table 8

Summary of Study Sample Concentrations for CA28-2GS-BF in Monkey Serum (SQ @ 7 mg/kg/day x 7 days)			
	CA28-2GS-BF Cone. (µg/mL)		
Timepoint	Animal 1	Animal 2	Animal 3
5 min	0	5.18	3.55
15 min	0	5.57	3.47

(continued)

Summary of Study Sample Concentrations for CA28-2GS-BF in Monkey Serum (SQ @ 7 mg/kg/day x 7 days)			
	CA28-2GS-BF Cone. (µg/mL)		
Timepoint	Animal 1	Animal 2	Animal 3
30 min	0	4.66	3.93
1 hr	0	5.41	3.56
4 hr	4.41	12.6	11.1
8 hr	32.0	15.3	21.5
Day 2	54.2	56.6	53.5
Day 3	135	117	122
Day 4	248	260	234
Day 5	398	316	311
Day 6	447	391	419
Day 7	564	412	448
Day 8	591	432	468
Day 9	596	423	455
Day 15	152	241	199

Example 8: Inhibition of Complement-Mediated Lysis of Red Blood Cells from Patients with PNH

[0332] A modified Ham's test is performed to measure the ability of compstatin analogs to inhibit complement-mediated lysis of red blood cells from patients with PNH in vitro. Complement is activated by acidified serum with added magnesium to lyse the PNH red cells. The incubation is performed for 90 minutes. The read out is flow cytometry for PNH red cells using standard markers. Heat inactivated serum is used as a control (produces no hemolysis). Acidified serum in the absence of added complement inhibitor produces maximum lysis. The experiment is performed with serial two-fold dilutions of compstatin analogs CA28, CA28-2, CA28-2CS, CA28-2CS-BD, CA28-2GS, CA28-2GS-BF, CA28-2HS, CA28-2HS-BF, CA28-2TS, CA28-2GS-BF, and CA28-3. The concentration of each compound required to fully block hemolysis in vitro is determined. Red blood cells are also stained for C3 fragment deposition using anti-C3 polyclonal antibodies that do not contain any bridge leading to agglutination (e.g., either Ab4214 or Ab14396, both commercially available FITC-conjugated Abcam, Cambridge, United Kingdom) in order to measure the ability of the compounds to inhibit deposition of C3 fragments on PNH red blood cells. Results are compared with those obtained with eculizumab using the same assays.

Example 9: Long-Acting Compstatin Analogs in Patients with PNH

[0333] A cohort of subjects diagnosed with PNH is divided into 4 groups. Subjects in Groups 1 and 2 are treated with intravenous administration of CA28-2 or CA28-3, respectively, at a dose of between 5 mg/kg and 20 mg/kg, at time intervals between 1 and 2 weeks. Optionally, treatment is started at more frequent time intervals and then reduced in frequency for maintenance therapy. Subjects in Group 3 are treated with eculizumab according to the recommended dosing regimen. Group 4 serves as a control (no complement inhibitor therapy). Intravascular hemolysis (based on LDH measurement and/or (51)Cr labeling of RBCs), reticulocytosis (an indicator of anemia), hematocrit, hemoglobin concentration in the blood, opsonization of red blood cells (deposition of products of C3 activation, such as C3b, on red blood cells, which may be detected using flow cytometry), PNH symptoms, transfusion requirements, thromboembolic events, haemolysis-associated nitric oxide depletion, measures of pulmonary hypertension, quality of life, and survival are monitored over time. Results are compared between groups and with historical data from control PNH patients obtained in clinical trials of eculizumab. An improvement in persistent anemia (e.g., as evidenced by reduced reticulocytosis, reduced evidence of hemolysis, increased hematocrit, increased hemoglobin), improved quality of life, reduced PNH symptoms, reduced transfusion requirements, reduced thromboembolic events, reduced haemolysis-associated nitric oxide depletion, reduced measures of pulmonary hypertension increased quality of life, and/or increased survival, in subjects receiving CA28-2 (Group 1) or CA28-3 (Group 2), as compared with subjects in Group 4 is indicative of efficacy.

Example 10: Long-Acting Compstatin Analogs in Patients with PNH

[0334] Example 9 is repeated with the modification that subjects are individuals with PNH who remain transfusion-dependent and/or continue to have a hemoglobin below a cutoff (such as 9.0 g/dL) despite treatment with eculizumab. Results are compared among groups.

Example 11: Long-Acting Compstatin Analogs in Patients with aHUS

[0335] A cohort of subjects diagnosed with aHUS is divided into 4 groups. Subjects in groups 1 and 2 are treated with intravenous administration of CA28-2 or CA28-3, respectively, at a dose of between 5 mg/kg and 20 mg/kg, at time intervals between 1 and 2 weeks. Optionally, treatment is started at more frequent time intervals and then reduced in frequency for maintenance therapy. Subjects in Group 3 are treated with eculizumab according to the recommended dosing regimen. Intravascular hemolysis (based on LDH measurement), opsonization of red blood cells (deposition of products of C3 activation, such as C3b, on red blood cells), aHUS symptoms, renal function, need for plasma exchange or dialysis, quality of life, and survival are monitored over time. Results are compared between groups and with historical data from control aHUS patients obtained in clinical trials of eculizumab. Reduced evidence of hemolysis, improved quality of life, reduced aHUS symptoms, reduced need for plasma exchange or dialysis, increased quality of life, and/or increased survival, in subjects receiving CA28-2 or CA28-3, as compared with subjects in group 4 are indicative of efficacy.

[0336] Example 12: Examples 8 - 11 are repeated using CA28-2GS-BF, CA28-2HS, CA28-2HS-BF, CA28-2TS, and CA28-2GS-TS-BF.

[0337] Example 14: Examples 9 - 12 are repeated using CA28-2GS-BF, CA28-2HS, CA28-2HS-BF, CA28-2TS, and CA28-2GS-TS-BF administered daily by subcutaneous injection.

[0338] Example 14: Examples 8-11 are repeated using additional long-acting compstatin analogs.

[0339] Example 15: Examples 8-11 are repeated using cell-reactive compstatin analogs.

[0340] Example 16: Complement Activation Inhibiting Activity of a Long-Acting Compstatin Analog

[0341] CA28 and CA28-AEEAc-Lys were synthesized as described above. CA28-2TS-BF was synthesized using a reactive bifunctional PEG of the TS type in terms of the NHS carboxylate attachment chemistry, which was linked to two molecules of CA28-AEEAc-Lys via the primary amine of the lysine side chain. The complement activation inhibitory activity of CA28 and CA28-2TS-BF was assessed by measuring the effect of the compounds on complement activation via the classical pathway and via the alternative pathways using standard complement inhibition assays. The protocol for the classical pathway activation assay is described in Example 1. The protocol for alternative pathway activation also measures C3b deposition in an ELISA format and is described below. C3b deposition monitored using this method is generated through complement activated by the alternative pathway by lipopolysaccharide (LPS). Briefly, 96-well plates are coated with LPS. Compound being tested (referred to as "drug") is added, followed by addition of plasma or serum as a source of complement, and incubated. This is followed by addition of anti-human C3 HRP-conjugated antibody. After an additional incubation, substrate is added and signal detected. Details of the protocol are as follows:

ELISA-based Assay for Alternative Complement Pathway Activation

Materials:

[0342]

- Ninety six-well ELISA plate (Corning 3590)
- LPS from *Salmonella typhosa* - Sigma L7136 (40ug/ml in PBS)
- BSA 1% in PBS - Calbiochem #126626 1/30 dilution
- Veronal Buffer + 10 mM MgCl₂ + 10 mM EGTA (VB-Mg EGTA)
- Human plasma (collected with Lepirudin at 5ug/ml final concentration)
- Anti-human C3 HRP-conjugated Ab (Poli to C3-HRP Ab, Cappel 55237)
- Tween-20 Wash Buffer (0.05% in PBS)
- TMB (Peroxidase substrate) - 1:1 mixture of BD 51-2607KC and 51-2606KC.
- 3M H₂SO₄
- Micro-plate Reader

Protocol:

[0343]

EP 3 660 033 B9

1. Add 50ul/well of LPS at 40ug/ml (in PBS)
2. Incubate for 2 hours at room temp
3. Remove by shaking and tapping the plate.
4. Block by adding 200ul of 1% BSA/PBS
5. Incubate for 1 h at room temp
6. Remove by shaking and tapping the plate
7. Add 50 ul VB-Mg EGTA to wells #2 to 12
8. Add 100ul of starting drug dilution (2x in VB-Mg EGTA) to well 1.
9. Serially dilute (1:2) the drug from wells 1 to 10 as follow

- a. Take 50ul of solution from the originating well
- b. Add this to the next well
- c. Mix by pipetting several times
- d. Repeat up to well #10

Note: from well #10 remove 50ul and discard.

10. Add 50ul of 2x plasma dilution to wells 1 to 11
11. Incubate for 1h
12. Wash twice with wash buffer
13. Add 50ul of 1/1000 dilution of C3-HRP Ab in 1% BSA/PBS
14. Incubate for 1h
15. Add 100ul of TMB to all wells
16. Incubate for 30min
17. Add 50 ul 3M H₂SO₄
18. Read the plate at 450nm

Formula for VB Mg EGTA

[0344]

Barbital	5 mM
NaCl	72.5 mM
MgCl ₂	10 mM
EGTA	10 mM
pH	7.3-7.4

Stock solutions:

Veronal Buffer (5X)

[0345]

	Prod #	MW	For 500ml
9 mM Sodium Barbitone	Sigma B0500	206.17	927 mg
15.5 mM diethylbarbituric acid	Sigma B0375	184.19	1.42 grams

Mg-Cl₂ (10X)

[0346]

	Prod #	MW	For 50 ml
100 mM MgCl ₂ ·6H ₂ O	Sigma M0250	203.30	1.00 gram

EGTA (10x)**[0347]**

	Prod #	MW	For 25 ml
100mM EGTA	Sigma E8145	468.3	1.17 grams

To prepare 20 ml of working buffer:**[0348]**

- Weight 84 mg NaCl
- Add 4ml of 5X VB
- Add 2ml of EDTA 10X
- Add 2ml MgCl 10X
- Adjust volume to 20 ml with H₂O
- Adjust pH to 7.4

Results

[0349] Figure 10(A) shows percent inhibition of classical complement activation inhibiting activity by CA28 and CA28-2TS-BF as a function of molar concentration of the compounds. Figure 10(B) shows percent inhibition of alternative complement activation inhibiting activity by CA28 and CA28-2TS-BF as a function of molar concentration of the compounds. Raw data are tabulated in Table 9 below (4 replicates of each condition). Based on the inhibition curves shown in the figures and underlying data, the complement inhibiting activity of CA28-2TS-BF is at least as great as that of CA28 on a molar basis within the experimental error of the assay. These results further confirm the suitability of long-acting compstatin analogs described herein, e.g., for therapeutic purposes.

Table 9
AP Inhibition
% Inhibition

Cone uM	CA28				CA28-2TS-BF			
25	90.07908	89.9353	90.65421	89.21639	90.25768	91.10484	90.68126	90.96365
12.5	88.92883	89.21639	89.21639	90.94177	90.11649	89.12814	89.26933	88.56336
6.25	87.05967	89.07261	88.20992	88.64127	88.28098	87.29263	87.43382	87.43382
3.125	85.62186	87.49101	87.49101	86.77211	84.04518	84.46877	85.17473	85.03353
1.5625	70.81236	81.30841	82.02732	70.38102	77.40911	69.5023	79.80939	81.08012
0.18725		58.01582	55.57153	57.15313	61.73668	66.39605	72.74974	61.3131
0.390625	18.04458	12.4371	23.65205	24.94608	56.79492	44.9347	56.65372	40.69891
0.1953	15.02516		15.31273	15.31273	22.34381	23.89693	26.01483	20.6495
0.09766	2.803741	14.30625	6.685844	5.823158	13.87222	6.953766	10.62479	0.3176956
0.0488	-1.365921	5.679367	0.790802	-2.803734	0.6000748	2.012009	0.3176956	-2.929749
0	-2.803734	-1.509697	1.078362	3.235085	0.6000748	7.094963	2.153198	-0.5294724

CP Inhibition
% Inhibition

Cone uM	CA28				CA28-2TS-BF			
25	83.89539	79.90365		81.00482	82.09877		81.32716	77.31482
12.5	78.66483	67.24019	81.69305	83.75774	83.48766	80.70988	78.54939	76.08025
6.25	84.58362	84.03304	80.45423	81.28011	81.94444	78.39507	79.93827	74.84568
3.125	83.62009	81.69305	79.90365	81.8307	79.93827	77.9329	77.46913	76.69753
1.5625	76.04955	76.60014	79.76601	78.94012	71.60493	70.52469	74.53703	75.611728
0.78125	71.50723	69.85547	73.98486	72.33311	72.37654	68.20988	72.0679	71.14198
0.390625	58.84377	72.05782	68.89195	63.11081	79.16666	70.37037	71.2963	62.19136
0.1953	42.60152	44.39091			66.51234		47.68519	50.92593

(continued)

CP Inhibition
% Inhibition

5	Cone uM	CA28				CA28-2TS-BF			
	0.09766	24.7075	24.15692	23.05575	35.03098	41.66667	35.03086	48.91975	42.12963
	0.0488	15.7605	12.59464	15.20992	27.04749	12.50001	26.23457	26.23457	23.61111
	0	-20.99105	7.088783	12.04405	1.858231	5.09259	-0.1543198	-0.9259262	-4.012352

Example 17: Pharmacokinetic Properties of Long-Acting Compstatin Analog Administered by the Intravenous or Subcutaneous Route

[0350] This Example describes determination of pharmacokinetic parameters of long-acting compstatin analog CA28-2TS-BF following administration to Cynomolgus monkeys with a single intravenous (IV) injection, single subcutaneous administration, or with once daily subcutaneous administration for seven days. CA28-2TS-BF was synthesized using a reactive bifunctional PEG of the TS type in terms of the NHS carboxylate attachment chemistry, which was linked to two molecules of CA28-AEEAc-Lys via the primary amine of the lysine side chain.

Dosing and Sample Collection

[0351] CA28-2TS-BF was administered to Cynomolgus monkeys at time 0 via intravenous injection into the saphenous vein or via single subcutaneous injection or repeat subcutaneous injection (once daily, for seven days). Six non-naive female Cynomolgus monkeys, age 2-5 years, ranging in weight from 2.6 to 3.9 kilograms, were used in this study (three per group). The animals were healthy at the start of the trial. The study was not blinded. Animals were supplied with water ad libitum and a commercial diet twice daily prior to initiation of the study. Food was supplied to the animals per facility SOP prior to the study. Animals were not fasted. Animals were dosed at 7 mg/kg via intravenous or subcutaneous administration at time 0 on the appropriate day(s). Dosing solution concentration was 3.5 mg/mL for IV administration and 25 mg/mL for subcutaneous administration. Dosing volume was 2 mL/kg for IV administration and 0.28 mL/kg for subcutaneous administration. A size 23G3/4 gauge needle was used for subcutaneous administration. The compound was administered in 5% dextrose in water.

[0352] Blood specimens (~0.5 - 1 mL) were collected from the femoral vein at the following timepoints: Day 1: Pre-dose, 5 min, 15 min, 30 min, 1 hour (h), 4 h, 8 h. Days 2 -9:0 min. Day 15: Final sample based on Day 1 dosing. Each blood sample was collected from the monkey's femoral vein via direct venipuncture and placed into a red top serum tube containing no anticoagulant, and kept at room temperature for at least 30 minutes. Blood samples were centrifuged at a temperature of 4°C at 3000 xg for 5 minutes. Samples were maintained chilled throughout processing. Serum samples were collected after centrifugation and placed into sample tubes. Samples were stored in a freezer set to maintain -60°C to -80°C. Serum samples and leftover dosing solutions were shipped frozen on dry ice for analysis.

[0353] The site of each subcutaneous administration was observed to see how fast the injection volume was absorbed and also to see if the formulation left behind a lump or fully went away. The dose sites for the animals receiving subcutaneous injections were observed in the evening of each dosing day. The dose site did not appear to have a lump and was fully absorbed by that time based on visual inspection. All animals were observed twice daily and showed normal activity throughout the study. No compound-related abnormalities were noted in the animals throughout the study.

[0354] Sample Analysis. Plasma samples obtained as described above were analyzed by LC/MS/MS leveraging CID (collision induced degradation) similarly to the method described in Example 3.

Results.

[0355] Serum concentrations vs time for CA28-2TS-BF when administered IV or subcutaneously as described above are plotted in Figure 11. The data points represent all PEGylated CA28 compound detected. CA28 data shown on Figure 11 are historical data obtained in a previous study in which CA28 was administered intravenously to Cynomolgus monkeys. In that study, CA28 was detected in samples using HPLC/MS.

[0356] A peak serum concentration of about 500 micrograms/mL was achieved by subcutaneous administration of CA28-2TS-BF once daily for 7 days. The terminal half-life of CA28-2TS-BF was approximately 8 days when administered either IV or by single subcutaneous injection. Raw data are provided in Tables 10(A) (IV administration) and 10(B) (subcutaneous administration) below. (In Figure 11 and Tables 10(A) and 10(B), the day of dosing is considered day 0).

Table 10(A)

CA28 (IV) CA28-2TS-BF (single dose IV)

	Time (days)	200 mg/kg	7 mg/kg		
5	0.0035		232	190	214
	0.01		216	190	209
	0.02	34	221	177	199
	0.042	17	211	175	183
10	0.167	9	190	152	185
	0.333	5	212	191	154
	1	1.5	180	130	150
	2		141	116	126
15	3		128	98.7	113
	4		114	89.1	95.8
	5		105	75	87.1
	6		95.1	67	74.4
	7		83.3	61.4	69.9
20	8		86	52.8	68.4
	14		51	30.8	39.7

Table 10(B)

CA28-2TS-BF (single dose SC) CA28-2TS-BF (7 x daily SC)

	Time (days)	7 mg/kg			7 mg/kg/day		
25	0.0035	BQL	BQL	BQL	BQL	BQL	BQL
	0.01	1.42	BQL	1.7	BQL	BQL	BQL
30	0.02	3.55	1.64	3.8	BQL	BQL	BQL
	0.042	6.1	3.46	7.05	2.32	1.14	2.44
	0.167	15	12.2	20.6	14.7	5.76	12.1
	0.333	25	25.8	32.9	32.9	18	25.8
35	1	70	76.5	76	80.5	80	66.5
	2	107	101	96.6	196	185	169
	3	111	103	99.8	391	286	292
	4	108	98.9	99.5	455	377	405
40	5	99.4	97.6	101	427	404	486
	6	86.8	87	81.6	490	483	568
	7	75.2	83.2	78.6	607	502	564
	8	67.5	73.4	12.2	495	481	570
	14	38.3	44.5	40.7	322	298	397

BQL = below quantification limit

[0357] As noted above, CA28-2TS-BF was synthesized using a reactive bifunctional PEG of the TS type, resulting in formation of a carbamate after reaction with the primary amine of lysine. CA28-2GS-BF was synthesized using a reactive bifunctional PEG of the GS type in terms of the NHS carboxylate attachment chemistry, resulting in formation of an amide after reaction with the primary amine of lysine. The compound also contains an ester linkage, which is absent in CA28-2TS-BF. It is notable that the terminal half-life of about 8 days achieved with CA28-2TS-BF in this experiment was considerably greater than that of CA28-2GS-BF, which was found to have a half-life of about 5 days in a similar experiment (see Example 8). **Example 18: Compstatin Analogs Inhibit C3 Deposition on Red Blood Cells of PNH Patients and Protects against Complement-mediated Lysis**

[0358] A modified Ham's test was performed to assess the ability of compstatin analogs to protect PNH RBCs from complement-mediated lysis. RBCs from a patient with PNH were exposed to acidified human serum (as a source of complement components) and magnesium (Mg^{2+} , needed for alternative pathway activation) in the absence of comple-

ment inhibitors or in the presence of varying amounts of compstatin analogs CA28 or CA28-2GS-BF. Exposure to heat inactivated human serum was used as a control representing no significant complement-mediated lysis as complement is inactivated by heat. Exposure to acidified human serum and magnesium (Mg^{2+}) in the absence of complement inhibitors (pane labeled Mg^{2+}) was used as a control representing maximum lysis.

[0359] Following incubation, cells were stained with antibodies to CD59 and C3d. Cd59 level permitted the classification of the PNH RBCs as Type I, Type II, or Type III. Staining for C3d, a product of C3 activation and cleavage was used as a marker of C3 and C3 activation product deposition (loading). Flow cytometric analysis was performed to assess CD59 and C3d on RBC surfaces and to quantify the percentages of Type I, Type II, and Type III cells present in various samples.

[0360] Results of a dilution experiment demonstrating the effect of different concentrations of CA28 on C3 deposition and cell percentages are shown in Fig. 12(A). Results of a dilution experiment demonstrating the effect of different concentrations CA28-2GS-BF on C3 deposition and cell percentages are shown in Fig. 12(B). The results are presented quantitatively in Table 11 below. Type I cells (shown in orange in Fig. 12) have normal levels of CD59. Type III cells (shown in blue in Fig. 12) have essentially no detectable CD59. These cells are very susceptible to complement-mediated lysis. Type II cells (shown in purple in Fig. 12) have reduced levels of CD59 as compared with normal or Type I cells and have an intermediate sensitivity to complement-mediated lysis. In the presence of complement activation Type III cells rapidly lyse. Reduction in or absence of lysis may be evidenced by an increased presence of Type III cells, as is evident as a higher percentage of Type III cells in the no lysis panel compared with the panel in (presence of Mg^{2+} (max. lysis)) in both Figures 12(A) and 12(B). In other words, there are relatively fewer Type III cells in the positive control than in the negative control. Type II cells may eventually lyse in the presence of activated complement but can accumulate a considerable amount of C3 activation products such as as C3d before they do so. Reduction in or absence of lysis may be evidenced by increased levels of C3 or C3 activation products on Type II cells, as is evident by comparing the level of C3d on Type II cells in the no lysis panel with the level of C3d on Type II cells in the max. lysis panel in both Figures 12(A) and 12(B). In other words, there is more C3d on cells in the max. lysis panel than in the no lysis panel. Type I cells have functional CD59, so they deactivate convertase and therefore do not accumulate as much C3d as Type II cells. However, the amount of C3d they accumulate can be used as a surrogate indicator for the amount of lysis of the more vulnerable cells (Type II and III). Thus, reduced C3d on Type I cells is indicative of protection against lysis. A shift in the relative percentages of Type I, II, and III cells from the percentages present in the max. lysis control panels (Mg^{2+}) towards the percentages present in the no lysis control panels (heat inactivated serum) is indicative of protection against complement-mediated lysis. These percentages are shown in the table below. The column labeled %C'3 in Table 11 refers to the percentage of cells deemed "positive" for presence of C3 and C3 activation products ("C3 loading"). As can be seen in Figures 12(A) and (B) and Table 11, CA28 and CA28-2GS-BF demonstrated similar protection of PNH red cell lysis over the concentrations tested, with virtually no C3 loading on PNH red cells at 100 micrograms/ml compound or higher concentrations. Note that the percentages of Type III, II, and I cells in the presence of 100 ug/ml or more compstatin analog were essentially the same as in the no lysis control, indicating complete protection from complement-mediated lysis as determined by this assay. Concentrations below 100 ug/ml but above 60 ug/ml, e.g., at least 70 ug/ml, at least 80 mg/ml, or at least 90 ug/ml, but below 100 ug/ml were not tested in this experiment but may also provide significant protection. 100 micrograms/ml CA28-2GS-BF represents a concentration of about 2.5 micromolar, which is readily achievable in vivo as described herein.

Table 11: Percentages and C3 loading of Type I, II, and III PNH RBCs in the absence or presence of compstatin analogs (concentrations in micrograms/ml are shown)

	Type III %	% C'3	Type II %	% C'3	Type I %	%C'3
Heat inactivated (no lysis)	37.09	0.29	51.79	1.22	11.12	0.09
Magnesium added (maximum lysis)	15.75	0.96	64.7	13.48	19.55	3.2
CA28 1	19.42	1.2	64.81	10.06	15.77	1.7
CA28 4	18.43	0.83	64.9	10.42	16.68	1.76
CA28 8	17.83	0.7	66	11.12	16.17	1.66
CA2815	22.11	0.96	62.9	8.63	15	1.93
CA28 25	20.53	0.75	64.51	9.3	14.96	1.84
CA28 100	37.96	0.1	51.5	0.92	10.64	0.08
CA28 500	37.29	0.09	52.06	0.83	10.64	0.14
CA28-2GS-BF 20	19.19	0.99	65.35	12.28	15.46	2.18
CA26-2GS-BF 40	15.15	1.21	68.96	15.12	15.89	2.84

(continued)

	Type III %	% C'3	Type II %	% C'3	Type I %	%C'3
CA28-2GS-BF 50	13.87	1.29	69.46	15.94	16.67	2.26
CA28-2GS-BF 60	17.94	1.44	66.97	13.04	15.09	2.05
CA28-2GS-BF 100	35.32	0.11	53.93	0.86	10.75	0.07
CA26-2GS-BF 200	37.43	0.02	51.99	0.26	10.59	0.02
CA2B-2GS-BF 500	37.87	0.05	51.87	0.3	10.26	0.04

Example 19: Effect of Compstatin Analog and Soliris on C3 Deposition on Red Blood Cells from PNH Patient

[0361] A similar experiment to that described in Example 18 was performed to further demonstrate the protective effect of compstatin analog CA28-2GS-BF and compare it with that of anti-C5 antibody Soliris. A modified Ham's assay as in Example 18 was performed using PNH RBCs incubated in the presence of activated complement either in the absence of complement inhibitor (left panel) or in the presence of Soliris (middle panel) or CA28-2GS-BF (50 ug/ml) (right panel). Flow cytometry was performed after antibody staining using antibodies against CD59 and C3d. Results are shown in Figure 13. In this figure, quadrant 1 (Q1) and quadrant 3 (Q3) represent Type III cells. Quadrant 2 (Q2) and quadrant 4 (Q4) represent Types I and II cells. Q1 and Q2 represent cells with a significant and abnormally high amount of C3 activation product (e.g., C3d) deposition. Q3 and Q4 represent cells without significant C3d deposition or somewhat elevated level (right portion of of Q4) but less so than Q2 cells. The percentages of cells in the different quadrants is presented below each panel in Figure 13 and in Table 12 below.

Table 12

	No Inhibitor			Eculizumab			CA28-2GS-BF	
Population	# Events	%Parent		# Events	%Parent		# Events	%Parent
Q1	233	0.09		90,146	36.79		23	0.01
Q2	7,992	3.22		9,609	3.92		18	0.01
Q3	9,853	3.97		4,591	1.87		153,187	61.54
Q4	230,241	92.72		140,689	57.42		95,713	38.45

[0362] As can be seen, in the absence of inhibitors the great majority of cells lie in Q4 (Type I or Type II with low levels of C3 activation product deposition). Type III cells would have mainly been lysed, so their percentages (Q1 and Q3) are low. Q2 cells that accumulate C3 deposition products eventually lyse, so their number stays relatively low. In the presence of eculizumab, Type III cells are protected from lysis at least initially, but accumulate C3 activation products (e.g., C3d) as shown by the high percentage of Q1 cells as compared with the no inhibitor panel (36.79% vs 0.09%). The relative proportion of Q2+Q4 cells (Type I and II) is lower as a result of the increased survival of Type III cells. However, it is evident that significant deposition of C3 activation products (e.g., C3d) occurs on Type III cells, which may lead eventually to lysis or to clearance (in vivo). PNH RBCs treated with CA28-2GS-BF (right panel) exhibit essentially no deposition of C3d regardless of whether they are Type I, II, or III, in contrast to the results with eculizumab. The percentage of cells in Q1 and Q2 is negligible. There is a dramatic increase in the percentage of Type III cells (61.55%) as compared with results with no inhibitor or with eculizumab, indicating (together with the lack of C3d deposition) enhanced protection from lysis by CA28-2GS-BF.

Claims

1. A compstatin analog comprising:
a linear PEG moiety having a molecular weight of 40 kD and coupled to each of two compstatin analog moieties;
wherein:

- i) each compstatin analog moiety is a CA28-AEEAc-Lys moiety;
- ii) the linear PEG moiety is coupled to the Lys side chain of each CA28-AEEAc-Lys moiety via an amide bond; and
- iii) each CA28-AEEAc-Lys moiety is Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-[NH-

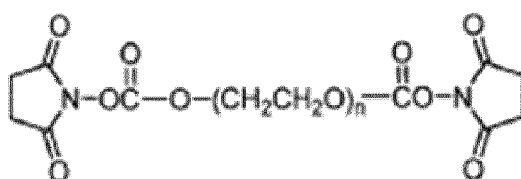
$\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{-C(=O)]-Lys-NH}_2$ (SEQ ID NO: 51); wherein the Cys* groups are joined by a disulfide bond.

2. A compstatin analog obtainable by coupling two CA28-AEEAc-Lys moieties to a bifunctional linear PEG moiety having a molecular weight of 40 kDa; wherein:

i) the bifunctional linear PEG moiety is coupled to the Lys side chain of each CA28-AEEAc-Lys moiety via an amide bond; and

ii) each CA28-AEEAc-Lys moiety is $\text{Ac-Ile-Cys}^*\text{-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys}^*\text{-Thr-[NH-CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{-C(=O)]-Lys-NH}_2$ (SEQ ID NO: 51); wherein the Cys* groups are joined by a disulfide bond.

3. A compstatin analog according to claim 2, wherein said bifunctional linear PEG moiety is of Formula IVa:



[Formula IVa]

4. A composition comprising a compstatin analog according to any one of claims 1 to 3, and a pharmaceutically acceptable carrier.
5. A compstatin analog according to any one of claims 1 to 3, or a composition according to claim 4, for use as a medicament.
6. A compstatin analog according to any one of claims 1 to 3, or a composition according to claim 4, for use in reducing the sensitivity of a cell or organ to complement-dependent damage.
7. A compstatin analog according to any one of claims 1 to 3, or a composition according to claim 4, for use in treating a complement-mediated disorder.
8. A compstatin analog according to any one of claims 1 to 3, or a composition according to claim 4, for use in treating or preventing age-related macular degeneration (AMD) or geographic atrophy (GA) in a subject in need thereof.
9. A compstatin analog for use or composition for use according to claim 8, wherein said use comprises administering said compstatin analog or said composition to the eye of said subject.
10. A compstatin analog for use or composition for use according to claim 9, wherein said use comprises administering said compstatin analog or said composition by intravitreal administration.
11. A compstatin analog according to any one of claims 1 to 3, or a composition according to claim 4, for use in treating transplant rejection; ischemia/reperfusion injury; haemolytic anemia, an autoimmune disease; neuropathic pain; membranoproliferative glomerulitis; neuromyelitis optica; spinal cord injury; asthma, paroxysmal nocturnal hemoglobinuria (PNH), or chronic obstructive pulmonary disease (COPD).
12. A compstatin analog for use or composition for use according to any one of claims 5 to 7 or 11, wherein said use comprises administering said compstatin analog or composition by subcutaneous or intravenous administration.

Patentansprüche

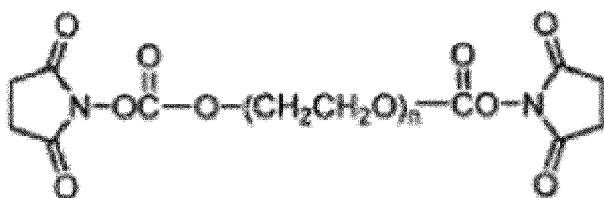
1. Compstatin-Analogon, das Folgendes umfasst:
eine lineare PEG-Einheit mit einem Molekulargewicht von 40 kD, die an jede der beiden analogen Compstatin-Einheiten gekoppelt ist; wobei:

- i) jede Compstatin-Analagon-Einheit eine CA28-AEEAc-Lys-Einheit ist;
 ii) die lineare PEG-Einheit über eine Amidbindung an die Lys-Seitenkette jeder CA28-AEEAc-Lys-Einheit gekoppelt ist; und
 iii) jede CA28-AEEAc-Lys-Einheit Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-[NH-CH₂CH₂OCH₂CH₂OCH₂-C(=O)]-Lys-NH₂ (SEQ ID NO: 51) ist; wobei die Cys*-Gruppen durch eine Disulfidbindung verbunden sind.

2. Compstatin-Analagon, das durch Kopplung von zwei CA28-AEEAc-Lys-Einheiten an eine bifunktionelle lineare PEG-Einheit mit einem Molekulargewicht von 40 kDa erhältlich ist; wobei:

- i) die bifunktionelle lineare PEG-Einheit über eine Amidbindung an die Lys-Seitenkette jeder CA28-AEEAc-Lys-Einheit gekoppelt ist; und
 ii) jede CA28-AEEAc-Lys-Einheit Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-[NH-CH₂CH₂OCH₂CH₂OCH₂-C(=O)]-Lys-NH₂ (SEQ ID NO: 51) ist; wobei die Cys*-Gruppen durch eine Disulfidbindung verbunden sind.

3. Compstatin-Analagon nach Anspruch 2, wobei die bifunktionelle lineare PEG-Einheit der Formel IVa entspricht:



[Formel IVa]

4. Zusammensetzung, die ein Compstatin-Analagon nach einem der Ansprüche 1 bis 3 und einen pharmazeutisch verträglichen Träger umfasst.
5. Compstatin-Analagon nach einem der Ansprüche 1 bis 3 oder eine Zusammensetzung nach Anspruch 4 zur Verwendung als Medikament.
6. Compstatin-Analagon nach einem der Ansprüche 1 bis 3 oder Zusammensetzung nach Anspruch 4 zur Verwendung bei der Verringerung der Empfindlichkeit einer Zelle oder eines Organs gegenüber komplementabhängigen Schäden.
7. Compstatin-Analagon nach einem der Ansprüche 1 bis 3 oder Zusammensetzung nach Anspruch 4 zur Verwendung bei der Behandlung einer Komplement-vermittelten Störung.
8. Compstatin-Analagon nach einem der Ansprüche 1 bis 3 oder Zusammensetzung nach Anspruch 4 zur Verwendung bei der Behandlung oder Verhinderung von altersbedingter Makuladegeneration (AMD) oder geografischer Atrophie (GA) bei einem Patienten, der dies benötigt.
9. Compstatin-Analagon zur Verwendung oder Zusammensetzung zur Verwendung nach Anspruch 8, wobei die Verwendung die Verabreichung des Compstatin-Analogons oder der Zusammensetzung an das Auge des Subjekts umfasst.
10. Compstatin-Analagon zur Verwendung oder Zusammensetzung zur Verwendung nach Anspruch 9, wobei die Verwendung die Verabreichung des Compstatin-Analogons oder der Zusammensetzung durch intravitreale Verabreichung umfasst.
11. Compstatin-Analagon nach einem der Ansprüche 1 bis 3 oder Zusammensetzung nach Anspruch 4 zur Verwendung bei der Behandlung von Transplantatabstoßung; Ischämie/Reperfusionsschaden; hämolytischer Anämie, einer Autoimmunerkrankung; neuropathischem Schmerz; membranproliferativer Glomerulitis; Neuromyelitis optica; Rückenmarksverletzung; Asthma, paroxysmaler nächtlicher Hämoglobinurie (PNH) oder chronisch obstruktiver Lungenerkrankung (Chronic Obstructive Pulmonary Disease, COPD).

12. Compstatin-Analogen zur Verwendung oder Zusammensetzung zur Verwendung nach einem der Ansprüche 5 bis 7 oder 11, wobei die Verwendung die Verabreichung des Compstatin-Analogons oder der Compstatin-Zusammensetzung durch subkutane oder intravenöse Verabreichung umfasst.

Revendications

1. Analogue de compstatine comprenant :

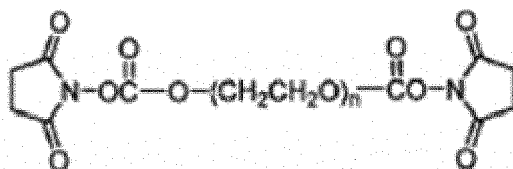
un fragment PEG linéaire ayant un poids moléculaire de 40 kD et couplé à chacun des deux fragments d'analogue de compstatine ; dans lequel :

- i) chaque fragment d'analogue de compstatine est un fragment CA28-AEEAc-Lys ;
- ii) le fragment PEG linéaire est couplé à la chaîne latérale Lys de chaque fragment CA28-AEEAc-Lys via une liaison amide ; et
- iii) chaque fragment CA28-AEEAc-Lys est Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-[NH-CH₂CH₂OCH₂CH₂OCH₂-C(=O)]-Lys-NH₂ (SEQ ID NO: 51) ; dans lequel les groupes Cys* sont liés par une liaison disulfure.

2. Analogue de compstatine pouvant être obtenu par couplage de deux fragments CA28-AEEAc-Lys à un fragment PEG linéaire bifonctionnel ayant un poids moléculaire de 40 kDa ; dans lequel :

- i) le fragment PEG linéaire bifonctionnel est couplé à la chaîne latérale Lys de chaque fragment CA28-AEEAc-Lys via une liaison amide ; et
- ii) chaque fragment CA28-AEEAc-Lys est Ac-Ile-Cys*-Val-(1Me)Trp-Gln-Asp-Trp-Gly-Ala-His-Arg-Cys*-Thr-[NH-CH₂CH₂OCH₂CH₂OCH₂-C(=O)]-Lys-NH₂ (SEQ ID NO: 51) ; dans lequel les groupes Cys* sont liés par une liaison disulfure.

3. Analogue de compstatine selon la revendication 2, dans lequel ledit fragment PEG linéaire bifonctionnel est de formule IVa :



[Formule IVa]

4. Composition comprenant un analogue de compstatine selon l'une quelconque des revendications 1 à 3, et un support pharmaceutiquement acceptable.

5. Analogue de compstatine selon l'une quelconque des revendications 1 à 3, ou une composition selon la revendication 4, à utiliser comme médicament.

6. Analogue de compstatine selon l'une quelconque des revendications 1 à 3, ou une composition selon la revendication 4, à utiliser pour réduire la sensibilité d'une cellule ou d'un organe aux dommages dépendant du complément.

7. Analogue de compstatine selon l'une quelconque des revendications 1 à 3, ou une composition selon la revendication 4, à utiliser dans le traitement d'un trouble médié par le complément.

8. Analogue de compstatine selon l'une quelconque des revendications 1 à 3, ou une composition selon la revendication 4, à utiliser dans le traitement ou la prévention de la dégénérescence maculaire liée à l'âge (DMLA) ou de l'atrophie géographique (GA) chez un sujet qui en a besoin.

9. Analogue de compstatine à utiliser ou composition à utiliser selon la revendication 8, dans laquelle ladite utilisation comprend l'administration dudit analogue de compstatine ou de ladite composition à l'œil dudit sujet.

10. Analogue de compstatine à utiliser ou composition à utiliser selon la revendication 9, dans laquelle ladite utilisation comprend l'administration dudit analogue de compstatine ou de ladite composition par administration intravitréenne.
11. Analogue de compstatine selon l'une quelconque des revendications 1 à 3, ou une composition selon la revendication 4, à utiliser dans le traitement de rejet de greffe ; lésion d'ischémie / de reperfusion ; l'anémie hémolytique, maladie auto-immune; douleur neuropathique ; glomérulite membranoproliférative ; neuromyéélite optique ; lésion de la moelle épinière ; d'asthme, d'hémoglobinurie paroxystique nocturne (HPN) ou de maladie pulmonaire obstructive chronique (MPOC).
12. Analogue de compstatine à utiliser ou composition à utiliser selon l'une quelconque des revendications 5 à 7 ou 11, dans lequel ladite utilisation comprend l'administration dudit analogue ou composition de compstatine par administration sous-cutanée ou intraveineuse.

Figure 1

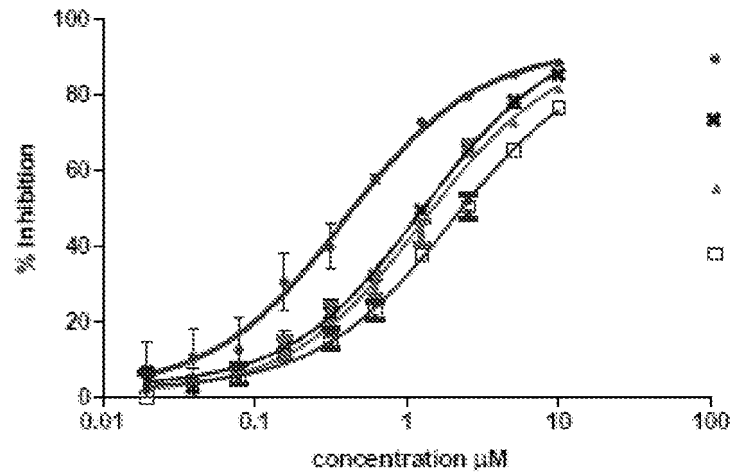


Figure 2

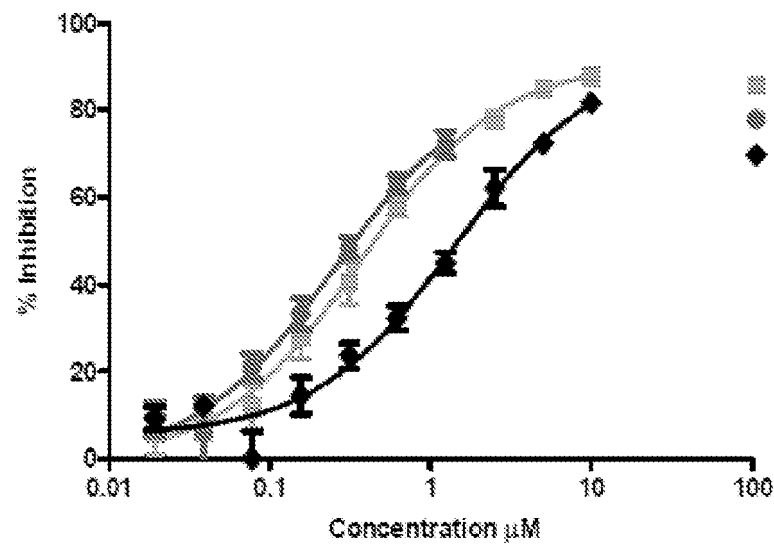


Figure 3

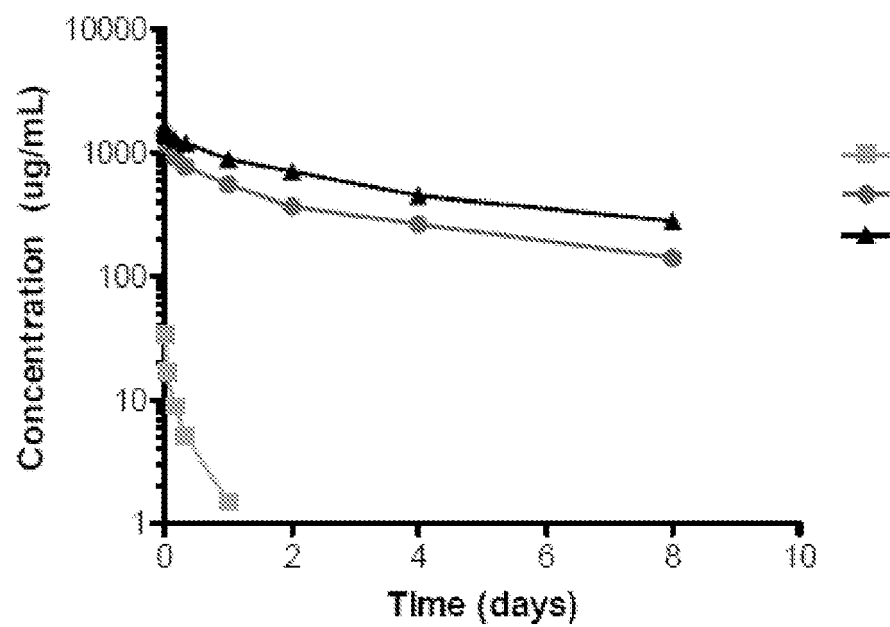


Figure 4

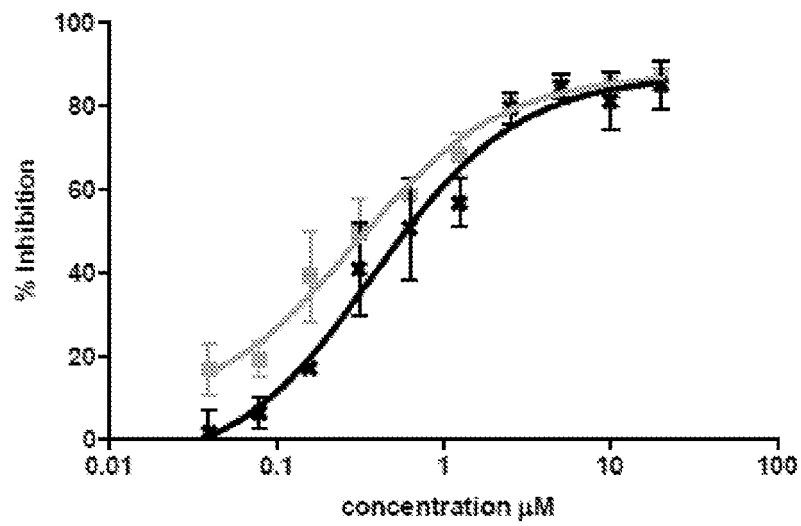


Figure 5

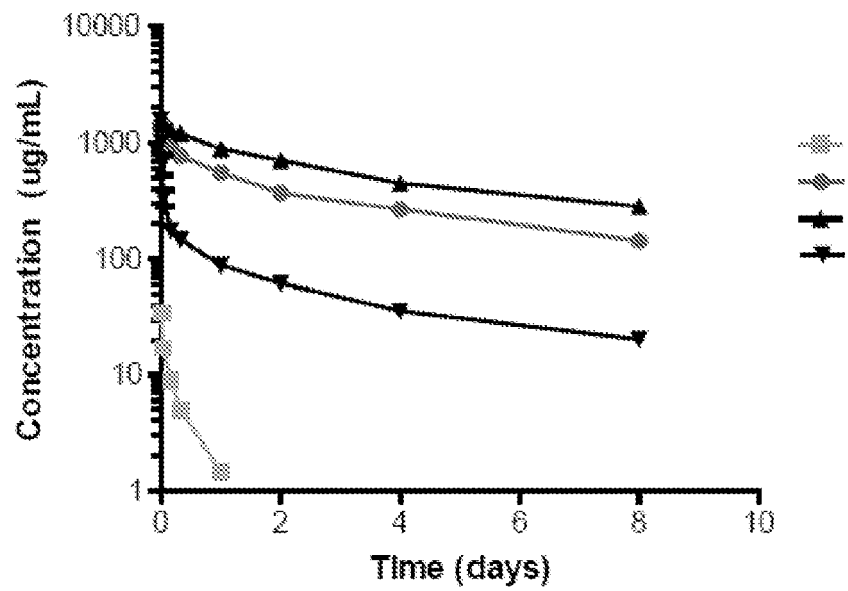


Figure 6

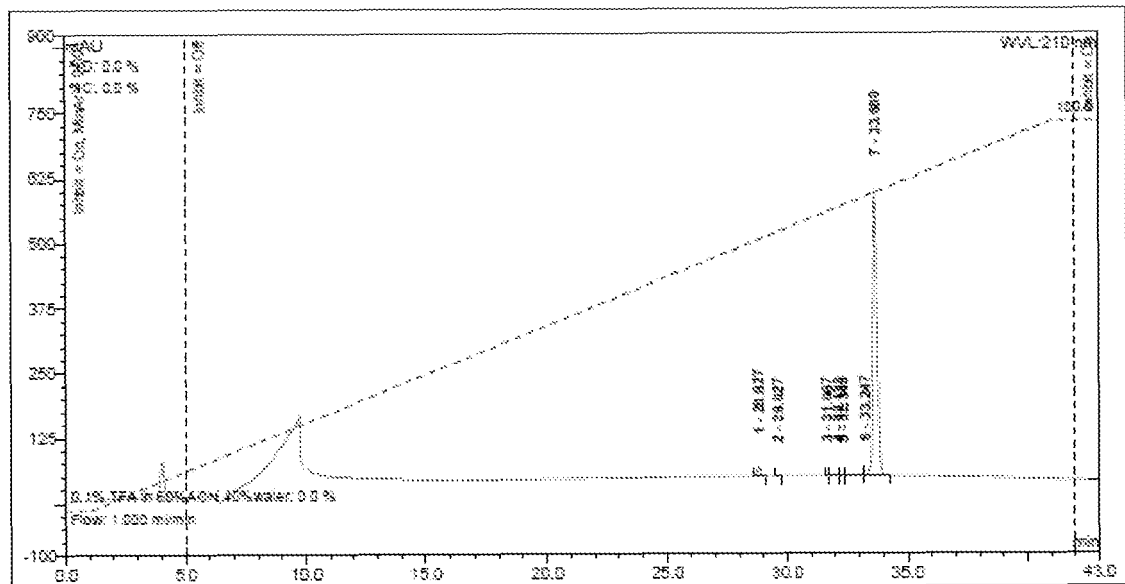


Figure 7

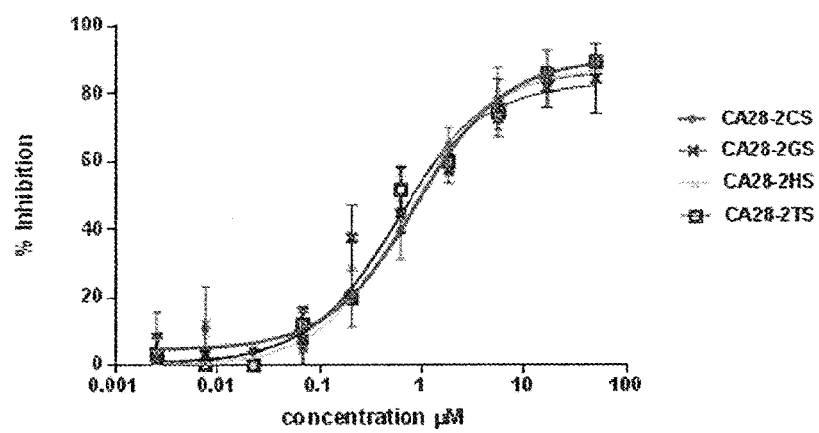


Figure 8

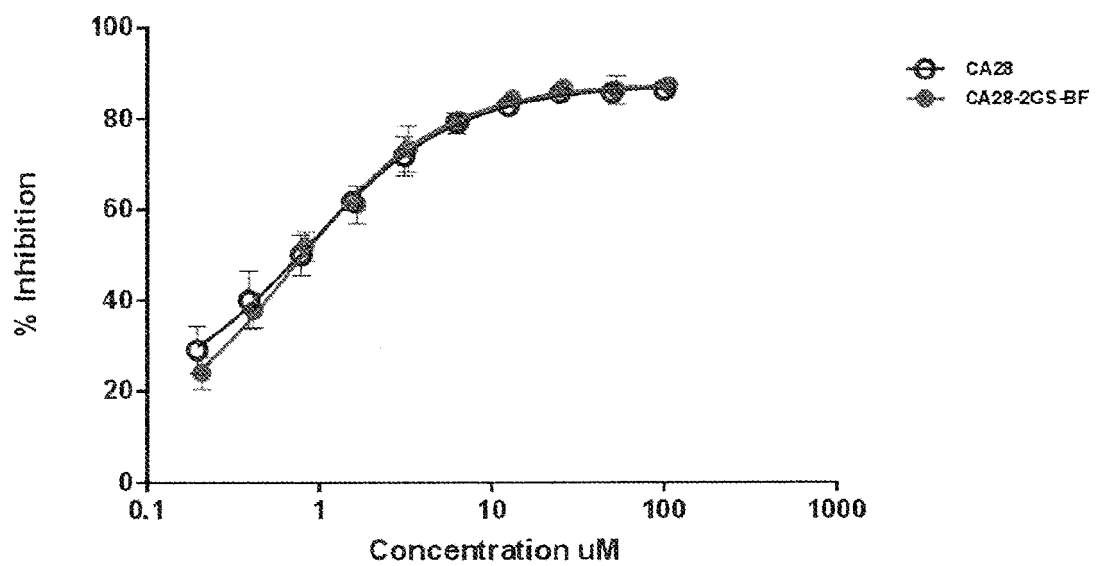
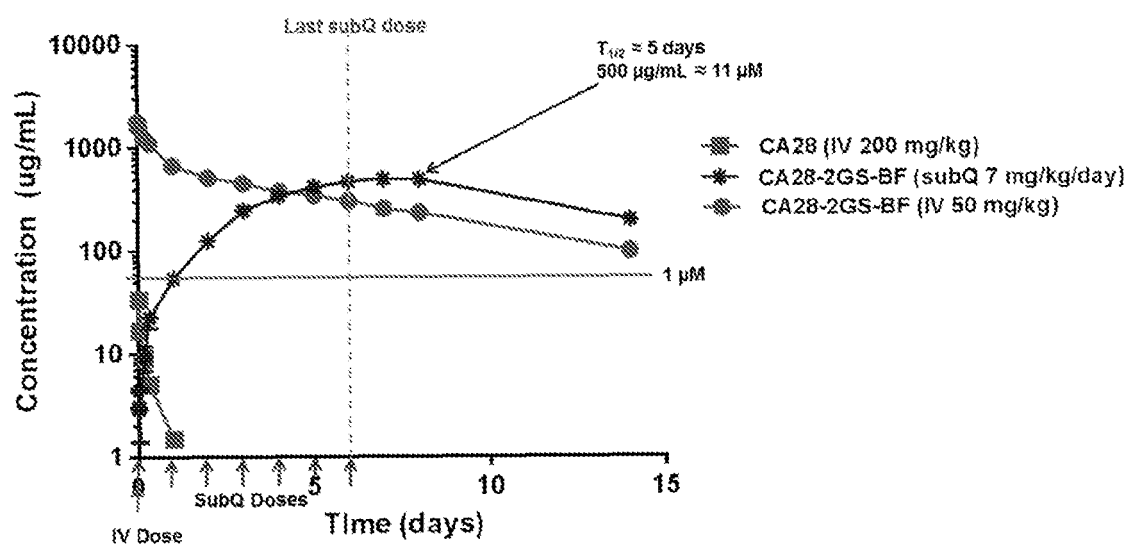


Figure 9



CA28-2TS-BF Activity

Classical Pathway Inhibition CA28 vs CA28-2TS-BF

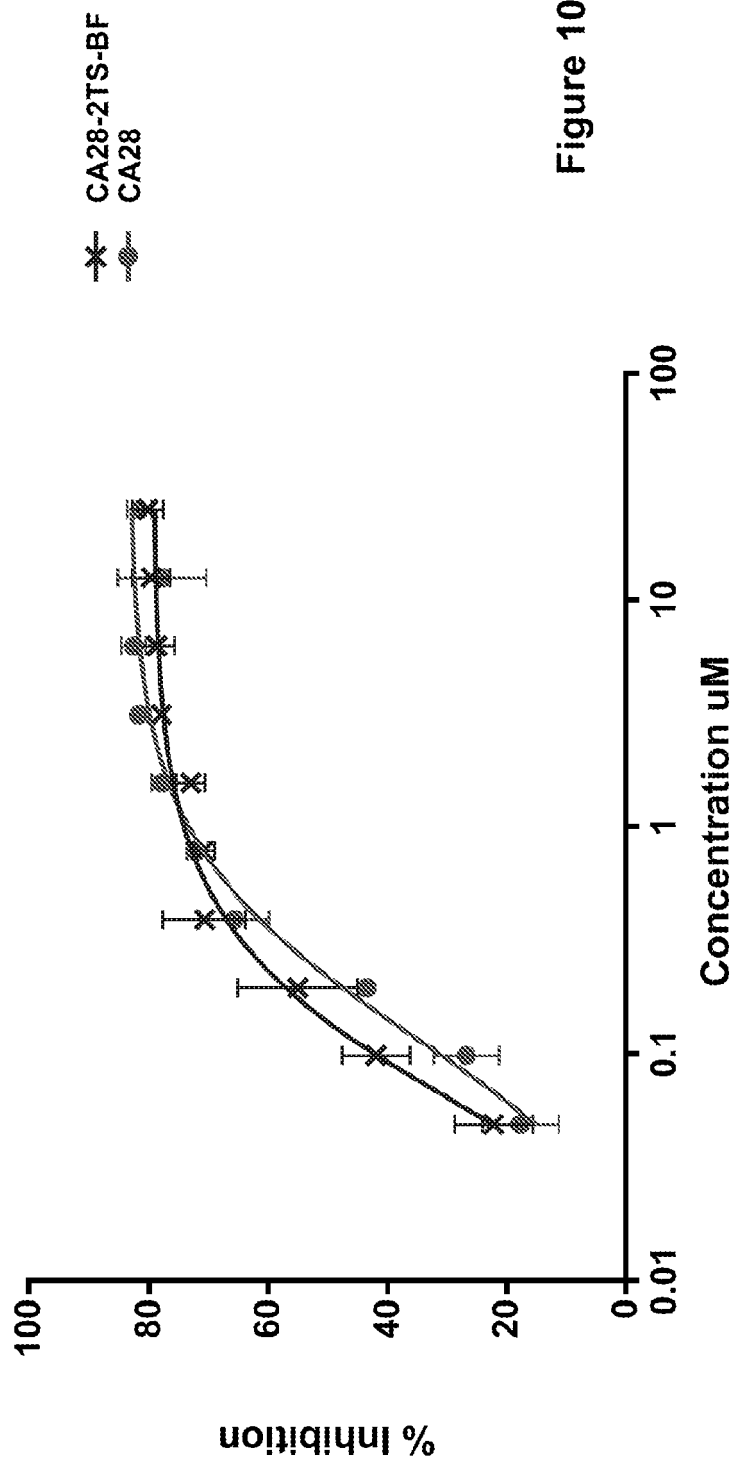


Figure 10(A)

CA28-2TS-BF Activity

Alternative Pathway Inhibition CA28 vs CA28-2TS-BF

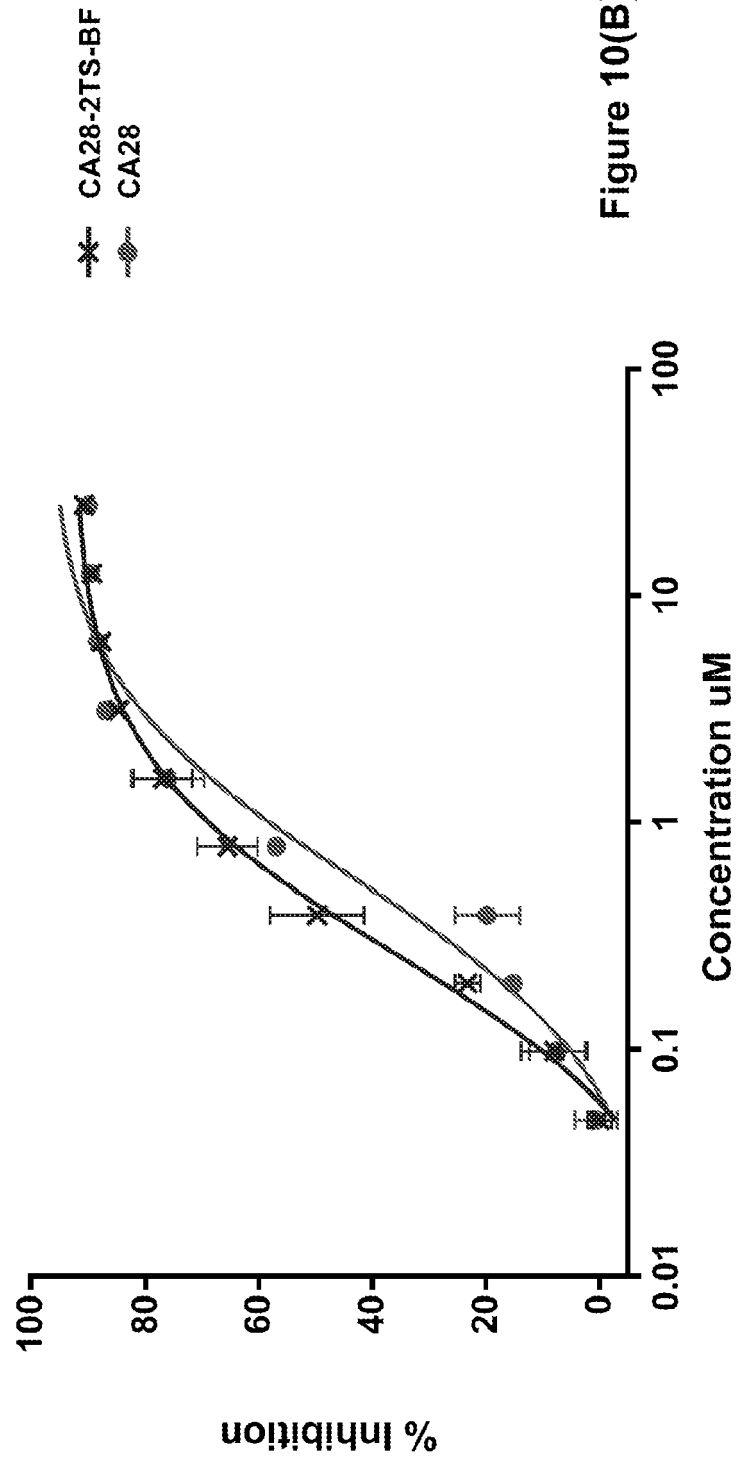


Figure 10(B)

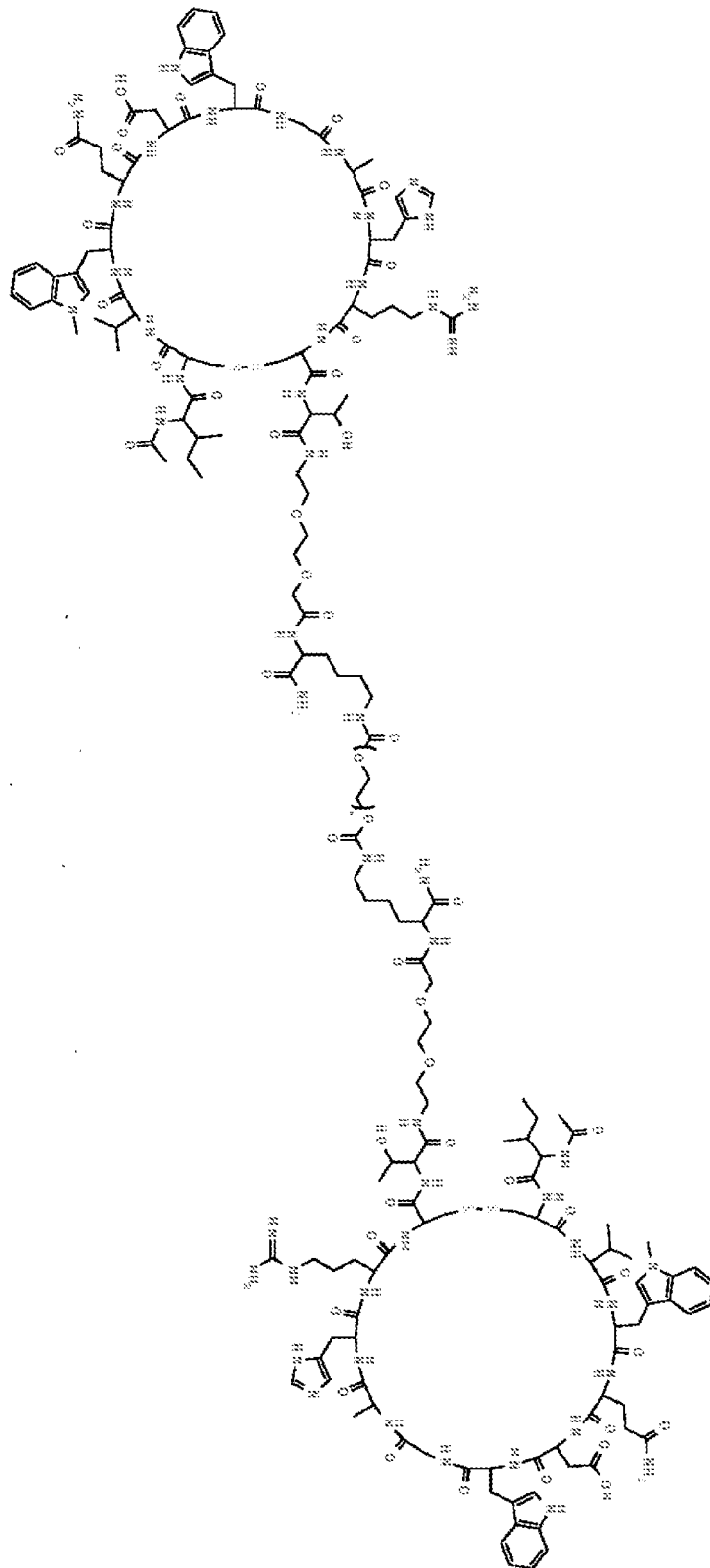


Figure 10(C)

CA28-2TS-BF IV vs CA28-2TS-BF SC vs CA28 PK ($\mu\text{g/mL}$)

CA28 IV vs CA28-2TS-BF IV and SC Pharmacokinetics

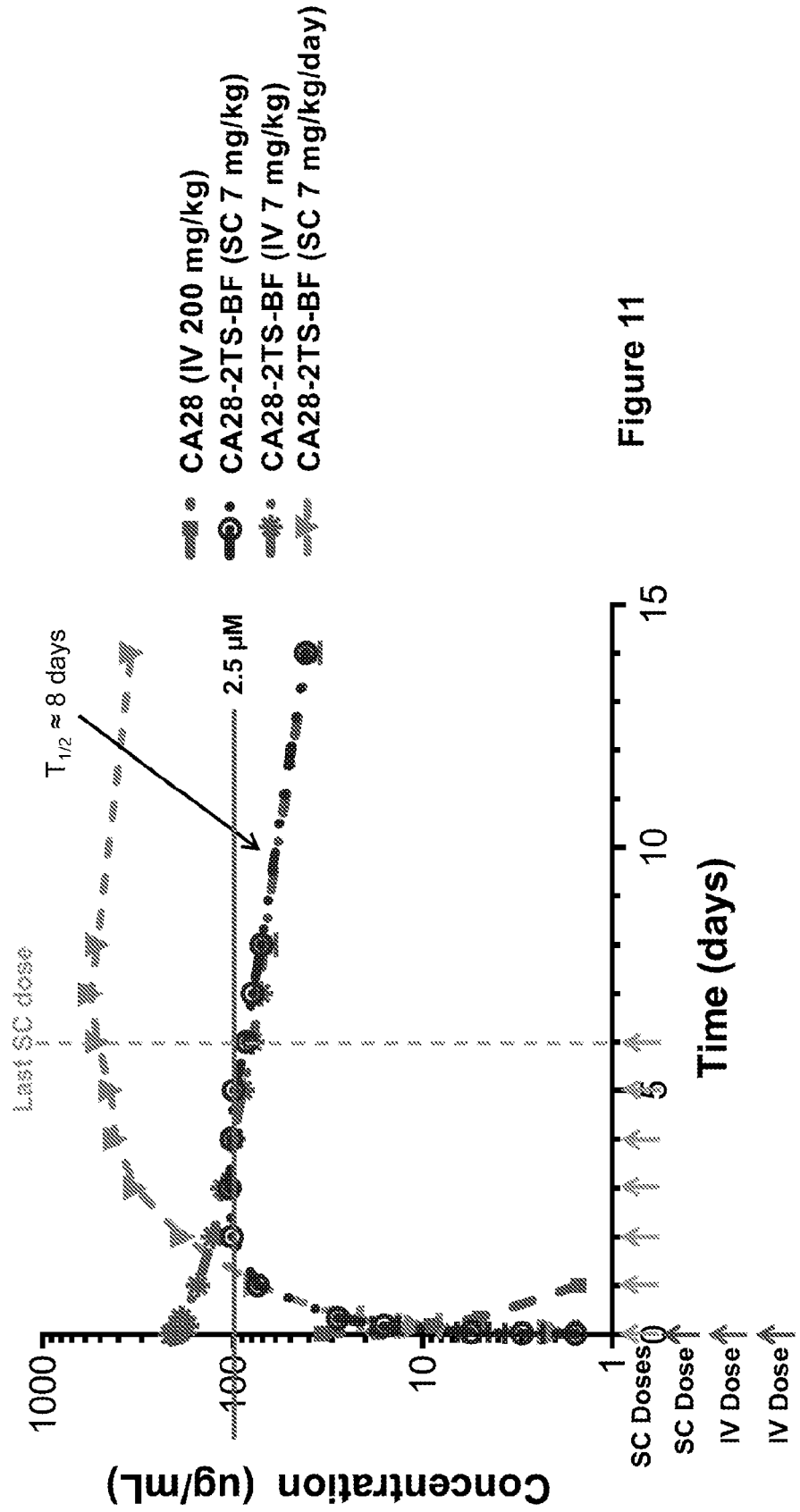


Figure 11

Dilution experiment – effect of CA28 on C3 loading

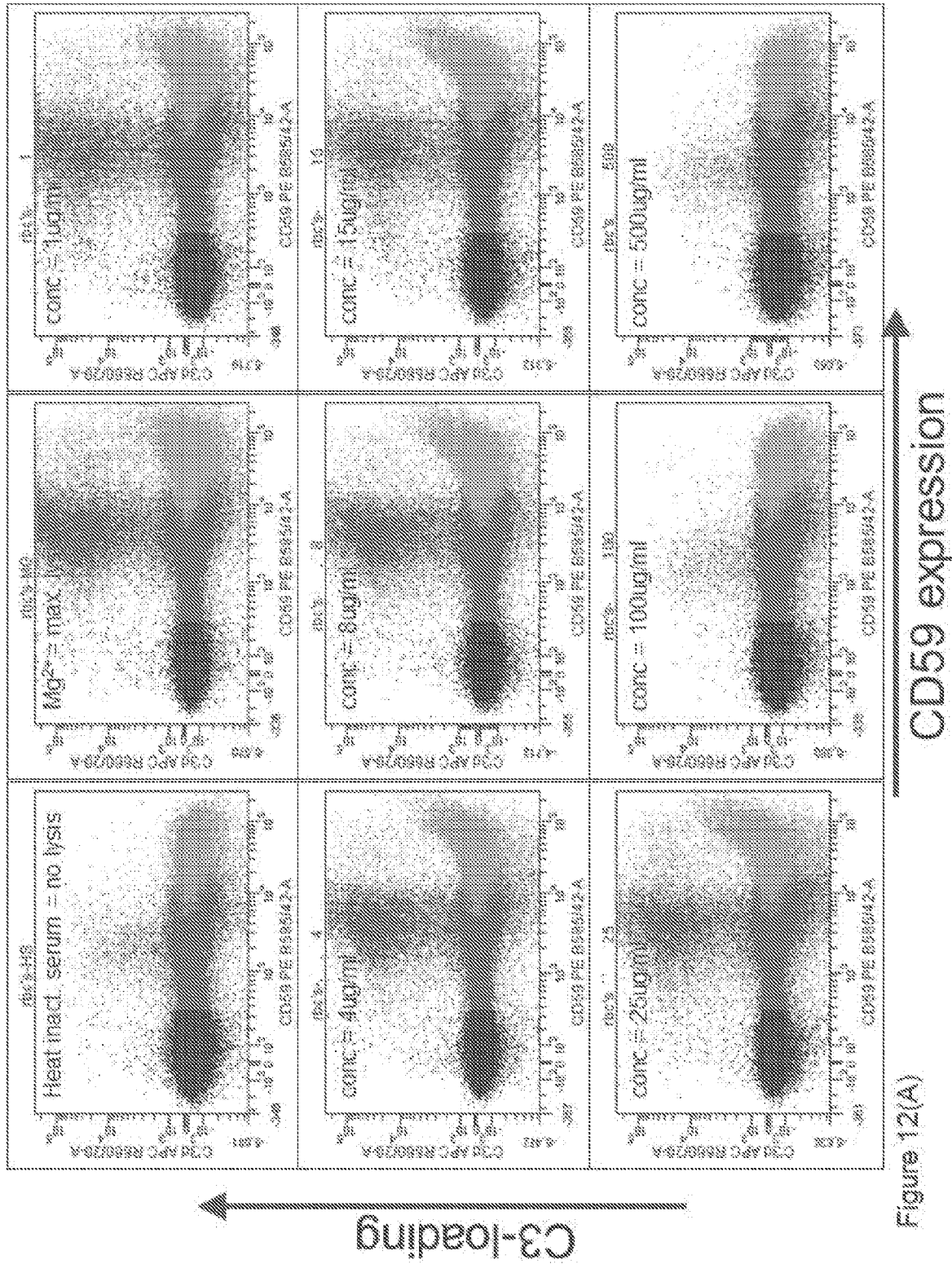


Figure 12(A)

Dilution experiment – effect of CA28-2GS-BF on C3 loading

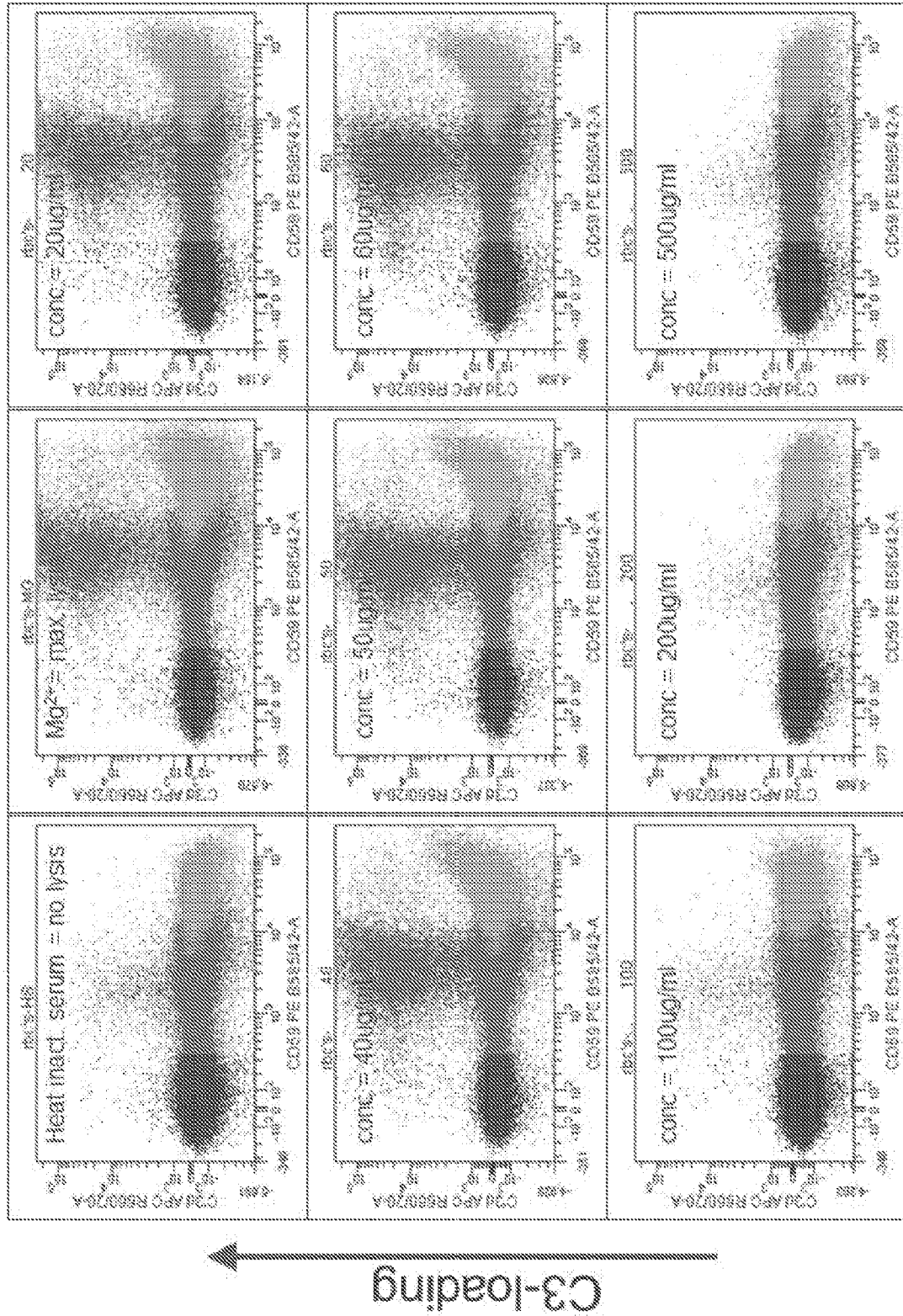


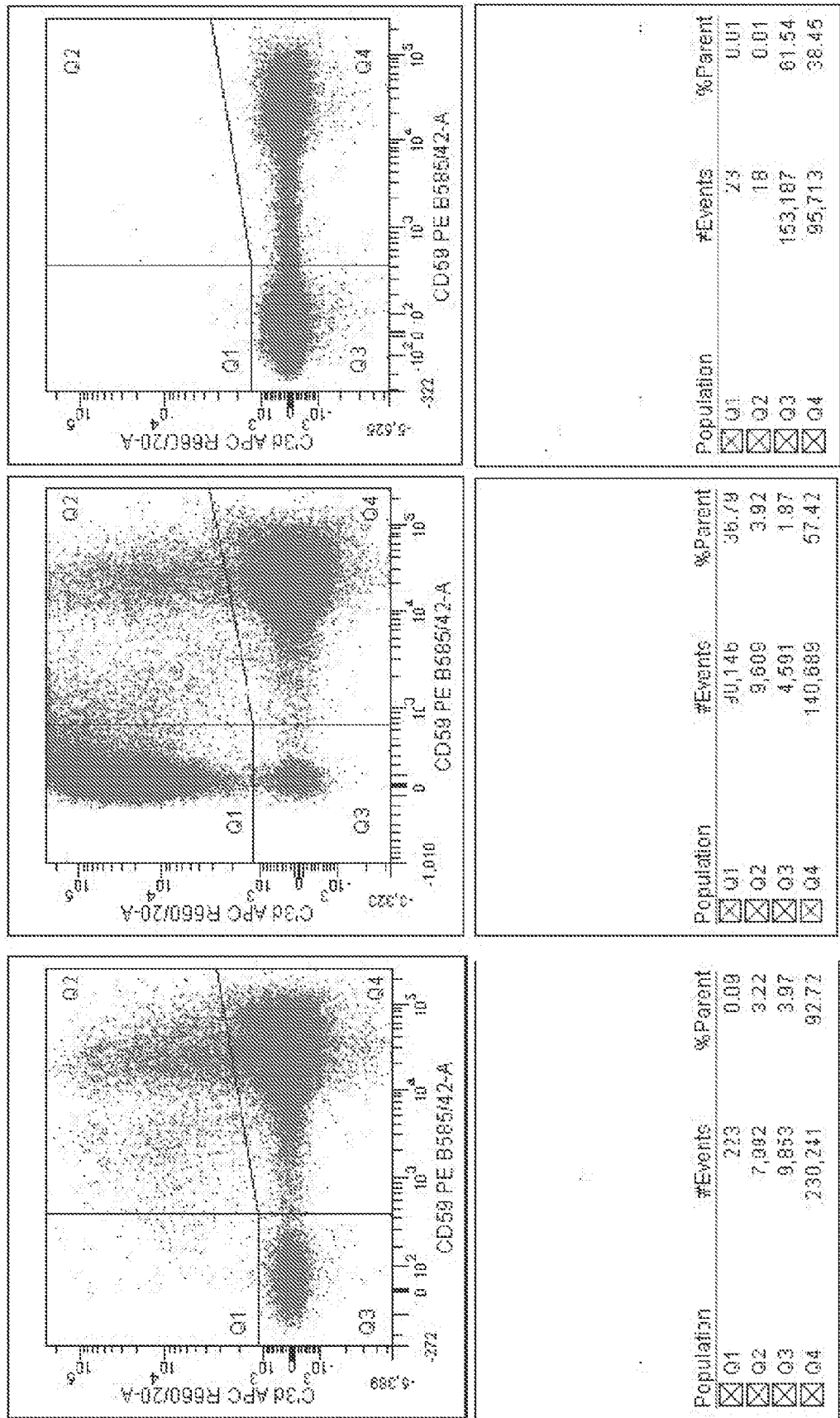
Figure 12(B)

CD59 expression

C3 loading PNH Red Cells during a modified Ham Test

Figure 13

No inhibitor Eculizumab (C5 inhibitor) CA28-2GS-BF(C3 inhibitor)



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