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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 December 2012 (20.12.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/174055 A1

(51) International Patent Classification:
A61K 38/10 (2006.01) *A61K 31/00* (2006.01)
A61K 38/12 (2006.01) *A61K 39/395* (2006.01)
A61K 38/17 (2006.01) *A61P 17/02* (2006.01)

(21) International Application Number:
PCT/US2012/042163

(22) International Filing Date:
13 June 2012 (13.06.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/496,248 13 June 2011 (13.06.2011) US

(71) Applicant *(for all designated States except US)*: **THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA** [US/US]; Center For Technology Transfer, 3160 Chestnut Street, Suite 200, Philadelphia, Pennsylvania 19104 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **LAMBRIS, John D.** [US/US]; 630 Pine Street, Philadelphia, Pennsylvania 19106 (US). **RAFAIL, Stavros** [GR/US]; Apt. 1804, 2101 Chestnut Street, Philadelphia, Pennsylvania 19103 (US).

(74) Agents: **REED, Janet E.** et al.; Potter Anderson & Corroon LLP, P.O. Box 951, 1313 N. Market Street, Wilmington, Delaware 19899-0951 (US).

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

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

[Continued on next page]

(54) Title: WOUND HEALING USING COMPLEMENT INHIBITORS

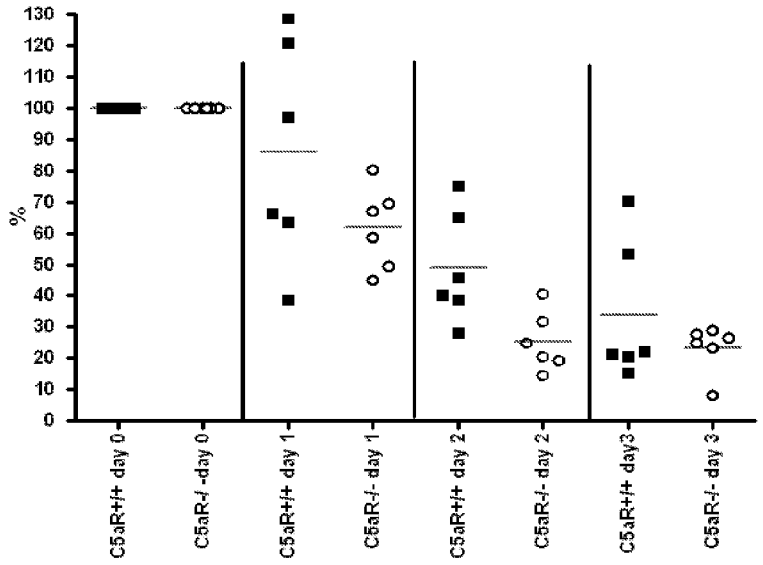

Fig. 1

(57) Abstract: Methods for promoting wound healing are disclosed. The methods involve administration of a complement inhibitor to inhibit complement activation, particularly through C3, C5 or C5a signaling. Pharmaceutical compositions comprising a complement inhibitor and at least one other agent for promoting wound healing are also disclosed

WO 2012/174055 A1

Published:

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

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WOUND HEALING USING COMPLEMENT INHIBITORS

Pursuant to 35 U.S.C. §202(c), it is acknowledged that the United States government may have certain rights in the invention described herein, which was made in part with funds from the National Institutes of Health under Grant Nos. GM-62134 and AI-068730.

FIELD OF THE INVENTION

This invention relates to the field of wound healing. Methods for stimulating wound healing are provided. The methods involve administration of a complement inhibitor to inhibit complement activation through at least C3, C5 or C5a receptor signaling to promote wound healing.

BACKGROUND OF THE INVENTION

Various publications, including patents, published applications, technical articles and scholarly articles are cited throughout the specification. Each cited publication is incorporated by reference herein, in its entirety. Full citations for publications not cited fully within the specification are set forth at the end of the specification.

A primary function of the skin is to serve as a protective barrier against the environment and pathogens. Loss of the integrity or excision of portions of the skin as a result of injury or illness initiates the process of cutaneous wound healing. Irregularities in this process can cause chronic wounds, which may lead to major disability or even death. Every year in the U.S., more than 1.25 million people suffer from burn injuries, and 6.5 million develop chronic skin ulcers caused by pressure, venous stasis, or diabetes mellitus[(1)]. The cost of chronic wound care has reached more than $3 billion annually in the U.S.[(2)] Wound healing of the skin or any other tissue or organ is a dynamic, intricate process that involves several phases, including coagulation, inflammation, maturation, and scar formation/remodeling. This order and the duration of each stage are vital for proper healing. Immune cells can greatly impact the repair process at each of these stages since their excessive presence can lead to chronic wounds and defective healing[(3)].

Shortly after wounding, the first cell population to arrive in large numbers is the platelets. Apart from their role in coagulation, platelets secrete a number of growth factors and other proteins that are involved in wound healing, including platelet-derived growth factor (PDGF), transforming growth factor b (TGF-b), fibroblast growth factor (FGF), epidermal growth factor (EGF), b-thromboglobulin, platelet factor 4 (PF4), platelet-derived angiogenesis factor (PDAF), serotonin, bradykinin, prostaglandins, prostacyclins, thromboxane, and histamine[4]. Platelet degranulation also initiates the complement cascade with the formation of C3a and C5a, which are potent anaphylatoxins that promote the release of histamine from basophils and mast cells[5]. Neutrophils arrive at the affected site soon after wounding and remain the major population in the area during the first few days. Their role is mainly bactericidal (through respiratory burst), but they also cleanse the wound of debris and damaged tissue. Neutrophils are attracted to the wounded site by fibronectin, growth factors, and kinins, among others[6]. Monocytes arrive at the wound site in response to factors released by platelets and other cells. Once they migrate from the periphery to the wound, they mature into macrophages, where they phagocytize bacteria and remove damaged tissue[7]. Mast cells secrete a number of inflammatory mediators and are important during inflammation, but recent studies have revealed that they are not required for proliferation.[8] In addition to the cells involved in innate immunity, the later stages of the healing process also involve cells of the adaptive immune response that target specific threats[9]. For example, T-helper and T-cytotoxic cells are found in the wounded area during the remodeling phase, and previous studies have shown that their depletion impairs wound healing[10]. Moreover, resident γδ T-cells of the dermis help establish homeostasis after injury, since they are actively involved in the attraction and activity of macrophages and the production of IGF1, keratinocyte growth factors (KGFs) and others.[11] Their role is so vital that their absence severely impairs wound healing[12].

In addition to their protective role, immune cells and the mediators they release are also important for the later stages of healing, such as the proliferative phase, including re-epithelialization and angiogenesis, and the remodeling phase, including scar formation, when fibroblasts increase in number and produce a scar in the repaired skin.[3]

Traditionally, cells of the immune system have been regarded as absolutely indispensable for proper wound healing. However, while immune cells are clearly essential for tissue clearance and preventing/fighting infection, the value of certain immune cells in other aspects of repair is now being challenged [3],[13]. One reason for this change in view is

the demonstration of the superior wound-healing capacity of fetal skin[14]. In this tissue, the standard series of phases is not followed, and immune cells are practically nonexistent during the healing process. Despite the lack of immune cell involvement, fetal wounds heal very rapidly and without scar formation, essentially regenerating normal skin in the wound area[14]. This view has been further strengthened as more questions have been raised by studies using adult animal models devoid of specific immune cell subtypes. More specifically, animals deficient or depleted of neutrophils, mast cells, or macrophages exhibit accelerated healing[15],[16]. Finally, depletion of neutrophils in a mouse model of chronic diabetic wounds also causes faster and improved healing.[17]

The complement system has traditionally been viewed as a component of innate immunity, yet recent research has suggested that complement components can also mediate novel, non-inflammatory functions and play critical roles in complex developmental and morphogenetic processes, such as coagulation,[18] hematopoiesis,[19] reproduction,[20] liver regeneration,[21] apoptosis,[22] and central nervous system development.[23] The complement system is based on various plasma proteins, pattern-recognition molecules, convertases and other proteases, regulators, and receptors for interactions with immune mediators.[5] The complement cascade can be either triggered by one of the three "traditional" pathways (classical, lectin, or alternative), which converge at the activation of C3 by specific convertases, or by a more recently described extrinsic pathway in which plasma proteases (e.g., thrombin, plasmin) act directly on C3 or C5. Activation of complement by all initiation methods leads to the production of the anaphylatoxins C3a and C5a and the membrane attack complex (MAC).

A major role of the complement cascade involves attracting, activating, and controlling innate and adaptive immune cells. C3a and C5a are powerful chemoattractants that guide neutrophils, monocytes, and macrophages to sites of complement activation[5]. Activated macrophages also produce C3 and participate in the complement-initiated phagocytosis of intruding entities and are also involved in the clearance of apoptotic and necrotic cells[28]. Moreover, C5a receptor (C5aR, or CD88) signaling in Toll-like receptor (TLR)-activated macrophages selectively inhibits the transcription of genes that encode the IL-12 cytokine family, which in turn drives the polarization and recruitment of Th1 cells.[29] Similarly, in spleen-derived dendritic cells (sDCs), C5aR activation plays an important role in the differentiation of naive CD4+ Th cells into Th1 or Th17 effector cells; blockade of C5aR in sDCs results in the expansion of T-regulatory cells (Treg).[30] Engagement of the

membrane-bound complement regulator CD46 differentially affects CD8+ T cell cytotoxicity, CD4+ T cell proliferation, and IL-2 and IL-10 production,[31]-[33] pointing to another role for complement components in regulating the immune response. C5a reacts with C5aR and C5L2 to induce the "cytokine storm" in sepsis.[34] Also, recent studies have shown that C1q can regulate the development of DCs from monocytes while affecting T-cell stimulation,[35] while others have shown that complement promotes Th17 differentiation with the participation of TLRs through C5aR signaling.[36] Finally, γδ T-cells express C5aR, and C5a itself contributes to the regulation of C5aR expression on these cells on a murine sepsis model[37]. Finally, keratinocytes, the major population of skin, express proteins and receptors for several complement components and regulators[38],[39]. Apart from the effect of complement on immune cells, recent work has also shown that complement is involved in other key aspects of wound healing, such as the effect of C5a on fibroblast migration and activation,[40] angiogenesis regulation,[41] and coagulation[18].

Despite the multifactorial role of complement in modulating this response in various diseases, little is known regarding its direct involvement in the regulation at the wound healing site. Advances in the art are needed to provide a practical link between the complement system and its modulation, for the purpose of promoting the wound healing process.

SUMMARY OF THE INVENTION

One aspect of the invention features a method for promoting wound healing in an individual, the method comprising: (a) identifying an individual who has been wounded, suffers a chronic wound, or will be wounded; and (b) administering to the individual a therapeutically effective amount of a complement inhibitor to the individual, wherein the complement inhibitor reduces or prevents complement activation, thereby promoting healing of the wound. The individual to be treated can be a human or a non-human animal.

In various embodiments, the complement inhibitor comprises one or more of a C3 inhibitor, a C3aR inhibitor, a C5a inhibitor, a C5aR inhibitor, a factor D inhibitor, a factor B inhibitor, a C4 inhibitor, a C1q inhibitor, or any combination thereof. In certain embodiments, the complement inhibitor is a C3 inhibitor. The C3 inhibitor can be selected from compstatin, a compstatin analog, a compstatin peptidomimetic, a compstatin derivative, or any combinations thereof, and may comprise SEQ ID NO:1, SEQ ID NO.:2, SEQ ID NO:3 or SEQ ID NO:4. In other embodiments, the complement inhibitor is a C5a inhibitor or a C5aR inhibitor. The C5a inhibitor or C5aR inhibitor can be selected from acetyl-Phe-[Orn-

Pro-D-cyclohexylalanine-Trp-Arg] (PMX-53), PMX-53 analogs, neutrazumab, TNX-558, eculizumab, pexelizumab or ARC1905, or any combination thereof. In other embodiments, the complement inhibitor is a C4 inhibitor.

The complement inhibitor can be administered systemically, or it can be administered locally or topically, or a combination approach may be utilized. The complement inhibitor may be administered together or concurrently with, or sequentially before or after, at least one other treatment for the wound.

Another aspect of the invention features a pharmaceutical composition for promoting wound healing in an individual, the pharmaceutical composition comprising one or more complement inhibitors and at least one other agent for treating the wound, in a pharmaceutically acceptable medium.

In various embodiments, the complement inhibitor comprises one or more of a C3 inhibitor, a C3aR inhibitor, a C5a inhibitor, a C5aR inhibitor, a factor D inhibitor, a factor B inhibitor, a C4 inhibitor, a C1q inhibitor, or any combination thereof. In certain embodiments, the complement inhibitor is a C3 inhibitor. The C3 inhibitor can be selected from compstatin, a compstatin analog, a compstatin peptidomimetic, a compstatin derivative, or any combinations thereof, and may comprise SEQ ID NO:1, SEQ ID NO.:2, SEQ ID NO:3 or SEQ ID NO:4. In other embodiments, the complement inhibitor is a C5a inhibitor or a C5aR inhibitor. The C5a inhibitor or C5aR inhibitor can be selected from acetyl-Phe-[Orn-Pro-D-cyclohexylalanine-Trp-Arg] (PMX-53), PMX-53 analogs, neutrazumab, TNX-558, eculizumab, pexelizumab or ARC1905, or any combination thereof. In other embodiments, the complement inhibitor is a C4 inhibitor.

The pharmaceutical composition can be formulated for systemic administration, or it can be formulated for local or topical administration.

Another aspect of the invention features kits for practicing the methods of the invention. The kits can comprise, among other things, various fixed or adjustable dosages forms of the pharmaceutical composition, devices and/or materials for administering the pharmaceutical compositions, and instructions for use of the compositions in the treatment of patients to promote wound healing.

Other features and advantages of the invention will be understood by reference to the drawings, detailed description and examples that follow.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Effect of complement component C3 on cutaneous wound healing. C3-/- mice and C3+/+ mice were wounded, and the wounded areas were compared over time. Wounded area is expressed as a percentage compared to the initial wound size (100%).

Figure 2. Effect of complement component C5aR on cutaneous wound healing. C5aR-/- mice and C5aR+/+ mice were wounded, and the wounded areas were compared over time. Wounded area is expressed as a percentage compared to the initial wound size (100%).

Figure 3. Effect of complement component C5 on cutaneous wound healing. C5-/- mice and C5+/+ mice were wounded, and the wounded areas were compared over time. Wounded area is expressed as a percentage compared to the initial wound size (100%).

Figure 4. C3 Reconstitution. C3 deficient mice were reconstituted with serum derived from C3 sufficient animals. The mice were wounded, and the wounded areas were compared over time.

Figure 5. Gene analysis of skin samples. Real-time RT PCR of 96 immune genes in the skin of C3-/-, C5aR-/- and their wildtype littermates was performed. Results are shown as -fold expression of complement-deficient samples compared to their respective wildtype littermates (one-fold). All genes below one were considered to be under-expressed. Of the 96 immune genes examined, only those with a statistically significant difference are shown; n=3 animals per group.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Various terms relating to the methods and other aspects of the present invention are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definition provided herein.

Definitions

Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry, and nucleic acid chemistry and hybridization are those well known and commonly employed in the art.

Standard techniques are used for nucleic acid and peptide synthesis. The techniques and procedures are generally performed according to conventional methods in the art and various general references (*e.g.*, Ausubel *et al.,* 2011, Current Protocols in Molecular Biology, John Wiley & Sons, NY), which are provided throughout this document.

The nomenclature used herein and the laboratory procedures used in analytical chemistry and organic syntheses described below are those well known and commonly employed in the art. Standard techniques or modifications thereof, are used for chemical syntheses and chemical analyses.

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

The singular form of a word includes the plural, and *vice versa*, unless the context clearly dictates otherwise. Thus, the references "a", "an", and "the" are generally inclusive of the plurals of the respective terms. For example, reference to "a compound" or "a method" includes a plurality of such "compounds" or "methods." Similarly, the words "comprise", "comprises", and "comprising" are to be interpreted inclusively rather than exclusively. Likewise the terms "include", "including" and "or" should all be construed to be inclusive, unless such a construction is clearly prohibited from the context.

The terms "comprising" or "including" are intended to include embodiments encompassed by the terms "consisting essentially of" and "consisting of". Similarly, the term "consisting essentially of" is intended to include embodiments encompassed by the term "consisting of".

Dosages expressed herein are in units per kilogram of body weight (*e.g.,* μg/kg or mg/kg) unless expressed otherwise.

Ranges are used herein in shorthand, to avoid having to list and describe each and every value within the range. Any appropriate value within the range is intended to be included in the present invention, as is the lower terminus and upper terminus, independent of each other.

The term "about" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or ±10%, in some embodiments ±5%, in some embodiments ±1%, and in some embodiments ±0.1% from the specified value, as such variations are appropriate to practice the disclosed methods or to make and used the disclosed compounds, compositions or articles of manufacture.

The term "antibody" refers to an immunoglobulin molecule that is able to bind specifically to a particular epitope on an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. The antibodies useful in the present invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, intracellular antibodies ("intrabodies"), Fv, Fab and $F(ab)_2$, as well as single chain antibodies (scFv), camelid antibodies and humanized antibodies (Harlow et al., 1999, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426).

A "complement inhibitor" is a molecule that prevents or reduces activation and/or propagation of the complement cascade that results in the formation of C3a or signaling through the C3a receptor, or C5a or signaling through the C5a receptor. A complement inhibitor can operate on one or more of the complement pathways, *i.e.,* classical, alternative or lectin pathway. A "C3 inhibitor" is a molecule or substance that prevents or reduces the cleavage of C3 into C3a and C3b. A "C5a inhibitor" is a molecule or substance that prevents or reduces the activity of C5a. A "C5aR inhibitor" is a molecule or substance that prevents or reduces the binding of C5a to the C5a receptor. A "C3aR inhibitor" is a molecule or substance that prevents or reduces binding of C3a to the C3a receptor. A "factor D inhibitor" is a molecule or substance that prevents or reduces the activity of Factor D. A "factor B inhibitor" is a molecule or substance that prevents or reduces the activity of factor B. A "C4 inhibitor" is a molecule or substance that prevents or reduces the cleavage of C4 into C4b and C4a. A "C1q inhibitor" is a molecule or substance that prevents or reduces C1q binding to antibody-antigen complexes, virions, infected cells, or other molecules to which C1q binds to initiate complement activation. Any of the complement inhibitors described herein may comprise antibodies or antibody fragments, as would be understood by the person of skill in the art.

A "subject", "individual" or "patient" refers to an animal of any species. In various embodiments, the animal is a mammal. In one embodiment, the mammal is a human. In another embodiment, the mammal is a non-human animal.

"Treating" refers to any indicia of success in the treatment or amelioration of the disease or condition, or promotion of the healing process. Treating can include, for example, reducing or alleviating the severity of one or more symptoms of the disease or condition, or it

can include reducing the frequency with which symptoms of a disease, defect, disorder, or adverse condition, and the like, are experienced by a patient, or it can include speeding, promoting or otherwise improving the healing process following injury to cells, tissues or organs. "Preventing" refers to the partial or complete prevention of the disease or condition in an individual or in a population, or in a part of the body, such as a cell, tissue or bodily fluid (*e.g.*, blood). "Promoting," such as promoting the healing process, refers to improving or accelerating the rate at which healing of a wounded cell, tissue or organ occurs. The term "prevention" does not establish a requirement for complete prevention of a disease or condition in the entirety of the treated population of individuals or cells, tissues or fluids of individuals. Nor does the term "promotion" establish a requirement that the healing of an entire population of injured cells, tissues or organs will be accelerated or improved.

A "prophylactic" treatment is a treatment administered to a subject (or sample) that does not exhibit signs of a disease or condition, or in advance of signs of the condition that are expected to manifest, such as symptoms of inflammation or stress after a trauma. This term may be used interchangeably with the term "preventing," again with the understanding that such prophylactic treatment or "prevention" does not establish a requirement for complete prevention of a disease in the entirety of the treated population of individuals or tissues, cells or bodily fluids.

As used herein, a "therapeutically effective amount" or simply an "effective amount" is the amount of a composition sufficient to provide a beneficial effect to the individual to whom the composition is administered, or who is otherwise treated using a method involving the composition.

As used herein, the term "wound" refers to a type of injury in which the integrity of a tissue, organ, membrane and the like is compromised, such as by a tear, abrasion, cut, puncture or burn, or where blunt force trauma causes a contusion (a closed wound). The term "wound" sometimes may be used interchangeably with the term "injury" herein. "Wound healing" and/or "wound repair" as used herein refer the intricate process by which a tissue or organ repairs itself after such injury. Since any tissue or organ can be wounded, the wound healing process can be observed in, for example, skin, muscle, adipose, bone, organs, connective tissue, and the like.

Description

The process of wound healing involves a continuous sequence of stages (coagulation, inflammation, proliferation, and maturation) and the concerted participation of numerous cell types associated with key biological activities (e.g., cell migration, proliferation, differentiation, and apoptosis). Major roles in healing are played by neutrophils, macrophages, fibroblasts, epidermal cells, and platelets, and a variety of cytokines and extracellular matrix proteins. The complement system, a key part of body's immune defense, has been linked to many of the processes and immune cells involved in wound healing. However, these associations have been demonstrated in other settings, but not in wound healing itself.

The present invention springs in part from the inventors' demonstration that modulation of key components of the complement system results in the acceleration of wound healing in an animal model system. For instance, as described in detail in Example 1, animals that were genetically deficient in three different complement components, C3, C5 and the C5a receptor (C5aR) demonstrated accelerated healing of cutaneous wounds, as compared with their similarly wounded wildtype counterparts. The role of C3 downregulation in accelerating the healing process was confirmed when the C3-deficient animals were reconstituted with serum from wildtype animals (containing C3) and their healing phenotype changed to that of the wildtype. Tissue analysis of healing wounds revealed that, in C3- and C5aR-deficient animals, the wounded areas contained decreased levels of inflammatory cells, while their healing proceeded much more rapidly than in wildtype animals. Gene expression analysis of C3- and C5aR-deficient animals and their wildtype counterparts revealed that the expression of inflammatory genes in the skin of complement-deficient appears to be generally suppressed, thereby contributing to the shortening of the post-wounding inflammatory phase. Thus, the downregulation or inhibition of complement activation, particularly at C3 and C5, or signaling through C5aR, has been shown to promote wound healing.

Accordingly, one aspect of the invention provides a method for promoting healing of wounds. The method comprises identifying or determining that an individual been wounded, either acutely or chronically, and administering a complement inhibitor to the individual to accelerate the wound healing process. Alternatively, the method comprises identifying an individual who will be wounded, such as by a surgical or dental procedure, and administering

a complement inhibitor to that individual to predispose the individual to accelerated wound healing once the wound is incurred.

For complement inhibition, any complement inhibitor may be utilized. Inhibitors of C3 or C5, or of C5a formation or activity may be used in the method of the invention. In one embodiment, the complement inhibitor is a C3 inhibitor. Preferably, the C3 inhibitor is compstatin or a compstatin analog, derivative, aptamer or peptidomimetic. Compstatin is a small molecular weight cyclic peptide having the sequence Ile-Cys-Val-Val-Gln-Asp-Trp-Gly-His-His-Arg-Cys-Thr (SEQ ID NO. 1). Examples of compstatin analogs, derivatives and peptidomimetics are described in the art. See, for instance, U.S. Pat. No. 6,319,897, U.S. Patent No. 7,888,323, U.S. Patent No. 7,989,589, WO/1999/013899, WO/2010/127336 and WO/2012/040259.

An exemplary compstatin analog comprises a peptide having a sequence: Xaa1 – Cys – Val – Xaa2 - Gln - Asp - Trp - Gly – Xaa3 - His - Arg – Cys – Xaa4 (SEQ ID NO. 2); wherein:

Xaa1 is Ile, Val, Leu, Ac-Ile, Ac-Val, Ac-Leu or a dipeptide comprising Gly-Ile;

Xaa2 is Trp or a peptidic or non-peptidic analog of Trp;

Xaa3 is His, Ala, Phe or Trp;

Xaa4 is L-Thr, D-Thr, Ile, Val, Gly, or a tripeptide comprising Thr-Ala-Asn, wherein a carboxy terminal –OH of any of the L-Thr, D-Thr, Ile, Val, Gly or Asn optionally is replaced by $–NH_2$; and the two Cys residues are joined by a disulfide bond. Xaa1 may be acetylated, for instance, Ac-Ile. Xaa2 may be a Trp analog comprising a substituted or unsubstituted aromatic ring component. Non-limiting examples include 2-naphthylalanine, 1-naphthylalanine, 2-indanylglycine carboxylic acid, dihydrotryptophan or benzoylphenylalanine.

Another exemplary compstatin analog comprises a peptide having a sequence: Xaa1 – Cys – Val – Xaa2 - Gln - Asp – Xaa3 - Gly – Xaa4 - His - Arg – Cys – Xaa5 (SEQ ID NO. 3); wherein:

Xaa1 is Ile, Val, Leu, Ac-Ile, Ac-Val, Ac-Leu or a dipeptide comprising Gly-Ile;

Xaa2 is Trp or an analog of Trp, wherein the analog of Trp has increased hydrophobic character as compared with Trp, with the proviso that, if Xaa3 is Trp, Xaa2 is the analog of Trp;

Xaa3 is Trp or an analog of Trp comprising a chemical modification to its indole ring wherein the chemical modification increases the hydrogen bond potential of the indole ring;

Xaa4 is His, Ala, Phe or Trp;

Xaa5 is L-Thr, D-Thr, Ile, Val, Gly, a dipeptide comprising Thr-Asn or Thr-Ala, or a tripeptide comprising Thr-Ala-Asn, wherein a carboxy terminal –OH of any of the L-Thr, D-Thr, Ile, Val, Gly or Asn optionally is replaced by $–NH_2$; and the two Cys residues are joined by a disulfide bond. The analog of Trp of Xaa2 may be a halogenated trpytophan, such as 5-fluoro-l-tryptophan or 6-fluoro-l-tryptophan. The Trp analog at Xaa2 may comprise a lower alkoxy or lower alkyl substituent at the 5 position, *e.g.*, 5-methoxytryptophan or 5-methyltryptophan. In other embodiments, the Trp analog at Xaa 2 comprises a lower alkyl or a lower alkenoyl substituent at the 1 position, with exemplary embodiments comprising 1-methyltryptophan or 1-formyltryptophan. In other embodiments, the analog of Trp of Xaa3 is a halogenated tryptophan such as 5-fluoro-l-tryptophan or 6-fluoro-l-tryptophan.

An exemplary compstatin analog of this type is Ac-I[CVW(Me)QDWGAHRCT]I-NH_2 (SEQ ID NO:4), which can be synthesized as described by Katragadda M, *et al.*, 2006, *J Med Chem*. 49: 4616-4622.

Another set of exemplary compstatin analogs features compstatin or any of the foregoing analogs, in which Gly at position 8 is modified to constrain the backbone conformation at that location. In one embodiment, the backbone is constrained by replacing the Gly at position 8 (Gly8) with Nα-methyl Gly.

Another set of exemplary compstatin analogs features compstatin or any of the foregoing analogs, in which the disulfide bond between C2 and C12 is replaced with a thioether bond, *e.g.*, to form a cystathionine compound such as a delta-cystathionine or a gamma-cystathionine.

Another set of exemplary compstatin analogs features compstatin or any of the foregoing analogs, further comprising an added or substituted N-terminal component that improves (1) the peptide's C3, C3b or C3c binding affinity, (2) the peptide's solubility in aqueous liquids, (3) the peptide's plasma stability and/or (4) the peptide's bioavailability, as compared with an unmodified compstatin peptide under equivalent conditions. Examples of such compounds include the compounds disclosed in WO/2010/127336 further comprising N-methyl isoleucine (SAR) or d-tyrosine-isoleucine (dTyr-Ile) at the N-terminus).

Other C3 inhibitors include vaccinia virus complement control protein (VCP) and antibodies that specifically bind C3 and prevent its cleavage.

Inhibition of C5a formation or activity may be accomplished in a variety of ways. For instance, C5a activity may be inhibited directly by preventing or significantly reducing

the binding of C5a to its receptor, C5aR. A number of C5aR inhibitors are known in the art. Acetyl-Phe-[Orn-Pro-D-cyclohexylalanine-Trp-Arg] (AcF[OPdChaWR]; PMX-53; Peptech) is a small cyclic hexapeptide that is a C5aR antagonist and is exemplified herein. Analogs of PMX-53 (e.g., PMX-201 and PMX-205) that also function as C5aR antagonists are also available (see for instance Proctor et al., 2006, *Adv Exp Med Biol.* 586:329-45 and U.S. Pat. Pub. No. 20060217530). Neutrazumab (G2 Therapies) binds to C5aR, thereby inhibiting binding of C5a to C5aR. Neutrazumab (G2 Therapies) binds to extracellular loops of C5aR and thereby inhibits the binding of C5a to C5aR. TNX-558 (Tanox) is an antibody that neutralized C5a by binding to C5a.

C5a activity may also be inhibited by reducing or preventing the formation of C5a. Thus, inhibition of any step in the complement cascade that contributes to the downstream formation of C5a is expected to be effective in practicing the invention. Formation of C5a may be inhibited directly by inhibiting the cleavage of C5 by C5-convertase. Eculizumab (Alexion Pharmaceuticals, Cheshire, CT) is an anti-C5 antibody that binds to C5 and prevents its cleavage into C5a and C5b. Pexelizumab, a scFv fragment of Eculizumab, has the same activity. Similarly, ARC1905 (Archemix), an anti-C5 aptamer, binds to and inhibits cleavage of C5, inhibiting the generation of C5b and C5a.

In another embodiment, formation of C5a is reduced or prevented through the use of a C3 inhibitor, as described above. In other embodiments, formation of C5a is reduced or prevented through the use of an inhibitor of complement activation prior to C3 cleavage, *e.g.,* in the classical or lectin pathways of complement activation. Non-limiting examples of such inhibitors include, but are not limited to: (1) factor D inhibitors such as diisopropyl fluorophosphates and TNX-234 (Tanox), (2) factor B inhibitors such as the anti-B antibody TA106 (Taligen Therapeutics), (3) C4 inhibitors (e.g., anti-C4 antibodies) and (4) C1q inhibitors (e.g., anti-C1q antibodies). Likewise, inhibitors of signaling *via* the C3a receptor are also contemplated as being useful in the present invention.

Antibodies useful in the present invention, such as antibodies that specifically bind to either C4, C3 or C5 and prevent cleavage, or antibodies that specifically bind to factor D, factor B, C1q, or the C3a or C5a receptor, can be made by the skilled artisan using methods known in the art. See, for instance, Harlow, et al. (1988, In: Antibodies, A Laboratory Manual, Cold Spring Harbor, NY), Tuszynski et al. (1988, Blood, 72:109-115), U.S. patent publication 2003/0224490, Queen et al. (U.S. Patent No. 6, 180,370), Wright et al., (1992, Critical Rev. in Immunol. 12(3,4):125-168), Gu et al. (1997, Thrombosis and Hematocyst

77(4):755-759) and Burton et al., (1994, Adv. Immunol. 57:191-280). Anti-C3 and anti-C5 antibodies are also commercially available.

The complement inhibitor can be administered immediately upon identifying the individual as a target candidate, i.e., the individual having been wounded, or carrying a chronic wound. Alternatively, complement inhibitors can be administered as a prophylactic measure, in the event of a planned wounding, such as surgery or other invasive procedure. Since wounding often occurs outside the setting of a health care facility, the complement inhibitor may be administered "in the field", for instance, at or near the location where the wounding occurred or during transport of the patient to a health care facility such as a hospital, clinic, or physician's office. Accordingly, the complement inhibitor can be administered any time from immediately following the wounding, to within minutes, or an hour, or several hours, or within 24 hours following occurrence of the wounding. Alternatively, the complement inhibitor can be administered once a patient has been diagnosed with a chronic wound, and such administration can continue until the chronic wound heals.

During the treatment period, a single dose or multiple doses of complement inhibitor can be administered, as would be understood by the skilled practitioner. For example, a sufficient dose (or multiple doses) of complement inhibitor can be administered to reduce complement activation in the individual to within e.g., 1, 2, or 5 times the average level in individuals who have not been wounded.

The skilled artisan will appreciate that numerous biomarkers of complement activation can be measured for the purpose of determining when to initiate or when to cease administration of complement inhibitor.

The complement inhibitors can be administered singly or in combination with one another. They may also be administered as part of a treatment regimen to promote wound healing. For example, complement inhibitors can be administered within the first hours after a wound has occurred or before, during or after operative or conventional wound treatment (e.g., stitching, skin grafting, bone setting or other surgical repair, debridement, cleansing, dressing and/or treatment with antibiotics, negative pressure therapy, hyperbaric therapy, laser treatment, and the like), once or several times within several days up to one week, or longer in the case of chronic wounds.

The invention encompasses the use of pharmaceutical compositions comprising a complement inhibitor to practice the methods of the invention. Such a pharmaceutical

composition may consist of the active ingredient alone, in a form suitable for administration to a subject, or the pharmaceutical composition may comprise the active ingredient and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The active ingredient may be present in the pharmaceutical composition in the form of a physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

The formulations of the pharmaceutical compositions may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single-or multi-does unit.

As used herein, the term "pharmaceutically-acceptable carrier" means a chemical composition with which a compstatin analog may be combined and which, following the combination, can be used to administer the comstatin analog to an individual.

As used herein, the term "physiologically acceptable" ester or salt means an ester or salt form of the active ingredient which is compatible with any other ingredients of the pharmaceutical composition, which is not deleterious to the subject to which the composition is to be administered.

The pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg and 100 mg/kg body weight as a single bolus, or in a repeated regimen, or a combination thereof as readily determined by the skilled artisan. In certain embodiments, the dosage comprises at least 0.1 mg/kg, or at least 0.2 mg/kg, or at least 0.3 mg/kg, or at least 0.4 mg/kg, or at least 0.5 mg/kg, or at least 0.6 mg/kg, or at least 0.7 mg/kg, or at least 0.8 mg/kg, or at least 0.9 mg/kg, or at least 1 mg/kg, or at least 2 mg/kg, or at least 3 mg/kg, or at least 4 mg/kg, or at least 5 mg/kg, or at least 6 mg/kg, or at least 7 mg/kg, or at least 8 mg/kg, or at least 9 mg/kg, or at least 10 mg/kg, or at least 15 mg/kg, or at least 20 mg/kg, or at least 25 mg/kg, or at least 30 mg/kg, or at least 35 mg/kg, or at least 40 mg/kg, or at least 45 mg/kg, or at least 50 mg/kg, or at least 55 mg/kg, or at least 60 mg/kg, or at least 65 mg/kg, or at least 70 m /kg, or at least 75 mg/kg, or at least 80 mg /kg, or at least 85 mg/kg, or at least 90 mg/kg, or at least 95 mg/kg, or at least 100 mg/kg, on a daily basis or on another suitable periodic regimen. In a particular embodiment, the dosage is between about 0.5 mg /kg and about 20 mg /kg, or between about 1 mg /kg and about 10 mg /kg, or between about 2 mg /kg and about 6 mg /kg.

In a particular embodiment, fixed dose formulations containing sufficient complement inhibitor to significantly inhibit complement activation in individuals of different size or maturity; e.g., a child or adult human, following a single administration (which may take the form of an IV bolus or infusion or, in the case of orally bioavailable agents, oral administration). For example, such formulations may be designed to reduce complement activation by between 50% and 99%, e.g., by at least 50%, 60%, 70%, 80% or 90%, relative to levels present prior to administration or relative to levels that would have been expected in the individual under the circumstances, in the absence of the complement inhibitor.

A single complement inhibitor may be administered, or two or more different complement inhibitors may be administered, in the practice of the method of the invention. In one embodiment of the invention, the method comprises administration of only a complement inhibitor. In other embodiments, other biologically active agents are administered in addition to the complement inhibitor in the method of the invention. Non-limiting examples of other biologically active agents useful in the invention include the variety of antibiotics used in conjunction with wound healing, as well as other pharmaceutical and non-pharmaceutical modulators; *e.g.,* cytokines and growth factors such as platelet derived growth factor (PDGF, bone morphogenetic proteins such as BMP-2 and -7, and growth differentiation factor (GDF) -5), steroids and other anti-inflammatory agents, bisphosphonates (such as aledronate), and cathepsin-K inhibitors, modulators of the coagulation cascade (such as factor XIII, Xa), and nutritional supplements such as aloe vera, Gotu kola, bromelain, cucurmin, arginine, glutamine, zinc, copper, pantothenic acid and vitamin C.

Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically or locally in oral solid formulations, parenteral, ophthalmic, suppository, aerosol, topical/transdermal or other similar formulations. Such pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer a complement inhibitor according to the methods of the invention.

Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in oral formulations, parenteral, ophthalmic (including intravitreal), suppository, aerosol, topical or other similar formulations. Such pharmaceutical compositions may contain pharmaceutically acceptable carriers and other ingredients known

to enhance and facilitate drug administration. Other formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer a complement inhibitor according to the methods of the invention.

As used herein, "oral administration" or "enteral administration" of a pharmaceutical composition includes any route of administration characterized by introduction into the gastrointestinal tract. Such administration includes feeding by mouth as well as orogastric or intragastric gavage. Such administration also may include sublingual, buccal or intranasal administration, among other routes known in the art.

Formulations of a pharmaceutical composition suitable for oral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, in a variety of dosage forms, including but not limited to pills, tablets, granules, powders, capsules, dispersions, suspensions, solutions, emulsions, gels and films, to name a few. Such dosage forms typically include carriers and excipients to facilitate formulation and delivery of the active ingredients.

The pharmaceutically acceptable carriers are selected from proteins, carbohydrates, lipids and combinations thereof. The active ingredients can be combined with the carrier in an appropriate diluent to form a solution or a suspension. Such liquid formulations can be viscous or non-viscous depending on the amount and the carrier used. The liquid formulations can be used directly or can be further formulated into an appropriate capsule, gel capsule or solid by methods know to those skilled in the art. Alternatively, solid formulations can be made by combining solid components. Such solid formulations can be used as a powder or formulated into granules, capsules, tablets or films any one of which can be made as a time release formulation.

Suitable proteins for use as carriers in oral dosage forms include milk proteins such as casein, sodium caseinate, whey, reduced lactose whey, whey protein concentrate, gelatin, soy protein (isolated), agar-agar, brown algae protein, red algae protein, bakers yeast extract and albumins. Suitable carbohydrates include celluloses such as methylcellulose, sodium carboxymethylcellulose, carboxymethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, cellulose acetate and ethyl cellulose, starches such as cornstarch, potato starch, tapioca starch, wheat starch, acid modified starch, pregelatinized starch and unmodified starch, alginates such as ammonium alginate, sodium alginate, and calcium alginate, glutens such as corn gluten and wheat gluten, gums such as acacia (gum Arabic), gum ghatti, guar gum, karaya gum (sterculia gum) and gum (tragacanth), insoluble

glucose isomerase enzyme preparations, sugars such as corn sugar, invert sugar, corn syrup, high fructose corn syrup, and sodium gluconate. Suitable lipids include tocopherols such as a-tocopherol acetate, oleic acid, oils such as coconut oil (refined), soybean oil (hydrogenated) and rapeseed oil, aluminum palmitate, dilauryl thiodipropionate, enzyme-modified lecithin, calcium stearate, enzyme-modified fats, glyceryl palmitostereate, lecithin, mono- and diglycerides, glycerin and waxes such as beeswax (yellow and white), candelilla wax and carnauba wax and vegetable oil.

For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active agent(s) suspended or dissolved in one or more carriers. Alternatively, the therapeutic agents can be formulated in a suitable lotion, cream, gel, or jelly containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Pastes, suspensions, emulsions, sprays, patches (*e.g.*, hydrogel patches), masks, and powders are other forms suitable for topical administration. Suitable carriers include, but are not limited to, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene, emulsifying wax, mineral or other oil, sorbitan monostearate, polysorbate 60, cetyl esters, wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. Any methods and materials suitable for preparing ointments, salves, gels, creams, etc., as generally known in the art may be used.

A formulation intended for topical administration to the skin can also include a skin penetration enhancer. The enhancer may increase effective transport of the agent into or through one or more layers of the epidermis or the dermis. A variety of delivery agents and approaches that enhance uptake and/or penetration of active agents into the skin are of use. In certain embodiments the delivery agent enhances penetration of the stratum corneum. Exemplary compounds include alpha-hydroxy acids, limonene, azone (AZ), lauryl alcohol (LA), other alcohols, isopropyl myristate (IPM), and the like.

Effective doses for topical application can be measured according to delivery at the selected topical site and need not depend on the body weight of the subject. For instance, topical doses can range between about 0.1 and 10,000 mg/dose for each location to be treated, *e.g.,* between about 0.5 and 5000 mg/dose or between 1 and 1000 mg/dose. Alternatively, concentrations of a therapeutic agent in a composition of the invention can range between about 0.001 and 100 mg of the therapeutic agent per milliliter of solution, *e.g.,* the concentration may be between 0.01 and 50 mg/ml or between 0.1 and 10 mg/ml.

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, intravenous, subcutaneous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. powder or granular) form for reconstitution with a suitable vehicle (e.g. sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable or infusible aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other useful parentally-administrable formulations include those comprising the active ingredient in microcrystalline form, in a liposomal preparation, in microbubbles for ultrasound-released delivery or as a component of a biodegradable polymer systems.

Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents including replacement pulmonary surfactants; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" that may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed., 1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, which is incorporated herein by reference.

In certain embodiments, the pharmaceutical composition may be a liquid formulation provided in a vial, a prefilled syringe, and the like. Such fixed dose formulations can be assembled into an article of manufacture containing a fixed dose of complement inhibitor in a convenient form for rapid administration to an individual. For instance, the formulation may be prepared for adding directly to an IV fluid solution.

The pharmaceutical compositions comprising complement inhibitors and/or other active agents or additional ingredients, can be conveniently packaged together in kits. Such kits comprise at least the complement inhibitor and instructions for its use in treating wounds. Such kits may also comprise the complement inhibitor and another treatment agent, along with instructions for their use. The kits may also comprise one or more of the diluents, excipients, carriers and other ingredients referred to above.

One embodiment features a kit containing at least one fixed dose formulation comprising a complement inhibitor and instructions for its use in treating a wounded patient. Also provided are kits containing multiple fixed dose formulations of a complement inhibitor, with at least two of the fixed dose formulations containing different amounts of the complement inhibitor. The different amounts can be selected to achieve a desired amount of complement inhibition depending on the size and/or maturity of the patient being treated; e.g., infants, children, and adults.

The following examples are provided to describe the invention in greater detail. They are intended to illustrate, not to limit, the invention.

Example 1

The roles of complement components C3, C5aR and C5 in the wound healing process were investigated.

Mice of strains C3+/+ (wildtype), C3-/-, C5aR+/+ (wildtype), C5aR-/-, C5+/+ (wildtype) and C5-/- were used. On day 0, two skin wounds were made in each animal with an Acu-Punch disposable skin biopsy punch (Acuderm, Inc., Fort Lauderdale, FL), per standard protocol. The mice were weighed and photographed daily. Wound size, measured as a percentage of the initial wound size, was recorded each day for three days.

C3-/- were reconstituted with serum from wildtype mice or from C3-/- mice, and the same process was performed.

Results are shown in Figures 1-4. C3 is the central component of all four initiation mechanisms (classical, lectin, alternative, and extrinsic pathways). The results with C3-/- mice strongly implicate complement in the early stages of the healing process (Fig. 1), since the lack of C3 leads to initial accelerated healing. This role for C3 was confirmed when C3-/- mice were reconstituted with serum from wildtype mice and their healing phenotype changed to that of the wildtype (Fig. 4). Similarly, C3-/- mice treated with serum derived from C3-/- animals showed the same accelerated healing as did the C3-/- mice alone, indicating the important role of C3 in the healing process. Experiments with mice lacking C5 and the C5a receptor (C5aR) similarly implicated C5a-dependent pathways (Fig. 2, Fig. 3) in control of wound healing.

Example 2

The involvement of the complement system at the level of proteins, immune cells, and the tissue environment, during wound healing was investigated.

For tissue analysis of wounds, tissues were obtained from C3+/+ (wildtype) and C3-/- mice 24 hours after wounding and subjected to Masson-trichome staining. The wildtype mice showed ulceration of the skin with intense, mostly acute inflammation in the surrounding dermis, subcutaneous fat and muscle. The inflamed area consisted mostly of neutrophils and macrophages. In contrast, in C3-/- mice the epidermis was relatively intact, with minimal

signs of inflammation in the dermis and surrounding tissue. A similar effect was observed in C5aR-/- mice as compared with wildtype animals. Thus, in complement-deficient mice (C3 and C5aR), the wounded area shows decreased levels of inflammatory cells, while its healing proceeds much more rapidly than in wildtype animals.

Skin samples from C3 or C5aR deficient mice were subjected to gene expression analysis. Real-time PCR of 96 immune genes in the skin of C3-/-, C5aR-/- and their wildtype littermates was performed. Results are shown in Figure 5. There was significant variation between the strains, indicating that the immune system in the skin of complement deficient mice was different from that of the wildtype mice, even before the wound was made, and the majority of the genes in the complement-deficient mice were underexpressed. Thus, it appears that the expression of inflammatory genes in the skin of complement-deficient mice is generally suppressed, thereby contributing to the shortening of the post-wounding inflammatory phase. These properties can be exploited as a "preventive" approach in patients about to undergo surgery or other invasive treatment.

Example 3 (Prophetic)

Those complement pathways or components identified by methods such as those described above to impede accelerated healing will be inhibited using appropriate therapeutic inhibitors, such as those shown in Table 1.

Table 1: Examples of Therapeutic Complement Inhibitors

Pathway component	Antagonist	Biological effect
C3	compstatin and analogs	Inhibition of classical, lectin, and alternative complement activation and, partly, of extrinsic
C1q	CBP2 peptide (LEQGENVFLQATLL)	Blockage of classical pathway
MBL	MBL inhibitory peptide (SFGSGFGGGY)	Blockage of lectin pathway
Factor B	Anti-Factor B (mAb 1378; IgG1)	Blockage of alternative pathway
C3a-C3aR axis	C3aRa (2,2-diphenylethoxyacetyl-arginine Anti-C3a (mAb 3/11; IgG2a)	Inhibition of C3aR signaling and anaphylactic, proinflammatory activities
C5	Anti-C5 (mAb BB5.1; IgG1)	Inhibition of C5a generation and formation of Terminal Attack Complex (C5b-9)
C5a-C5aR axis	C5aRa (PMX-53)	Inhibition of C5aR signaling

	Anti-C5a mAb $A8_{(\Delta 71\text{-}73)}$; dual C5aR/C5L2 inhibitor	and chemotactic, anaphylactic, proinflammatory activities
C5a-C5L2 axis	$A8_{(\Delta 71\text{-}73)}$; dual C5aR/C5L2 inhibitor Anti-C5L2 polyclonal antibody	Blockage of C5L2 that has a pro-inflammatory role in sepsis

Administration of complement inhibitors for wound healing is contemplated to be short-term in most situations. Since treatment with complement inhibitors does not affect long-term immunity, complement therapeutics provides an advantageous weaponry against various forms of impaired wound healing.

Selective targeting of complement components involved in the different stages of healing will be examined. One target population includes patients who are already wounded and are thus in an initiated stage of the healing process where a preventing infections is a priority. Another target population includes patients with chronic wounds, where inflammatory stages are prolonged and need to be treated in order to proceed to later healing stages. Another target population includes patients about to be wounded, such as patients scheduled to undergo surgery or other invasive procedure.

Depending on their bioavailability, the compounds will be administered either systemically or topically. A straightforward model of wound healing, e.g., based on simple wounding and healing of wildtype animals in the presence and absence of complement inhibitor, will be implemented. Moreover, in order to apply our findings to a clinically relevant model of chronic wound healing, we will use these therapeutic compounds in an established diabetes model (leptin receptor-deficient mice, which have chronic wounds). Further, in accordance with the preliminary PCR data set forth in the Example above, we will "preventively" treat wildtype animals in order to mimic the immune gene expression of the knockout mice; this suppressed gene expression at baseline level is expected to affect the subsequent wounding.

References:

1. Brigham PA, McLoughlin E. Burn incidence and medical care use in the United States: estimates, trends, and data sources. J Burn Care Rehabil 1996 Apr;17(2):95-107.

2. Bergan JJ, Schmid-Schönbein GW, Smith PDC, Nicolaides AN, Boisseau MR, Eklof B. Chronic venous disease. N. Engl. J. Med 2006 Aug;355(5):488-498.

3. Wilgus TA. Immune cells in the healing skin wound: influential players at each stage of repair. Pharmacol. Res 2008 Aug;58(2):112-116.

4. Nurden AT, Nurden P, Sanchez M, Andia I, Anitua E. Platelets and wound healing. Front. Biosci 2008;13:3532-3548.

5. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat. Immunol 2010 Sep;11(9):785-797.

6. Kim M-H, Liu W, Borjesson DL, Curry F-RE, Miller LS, Cheung AL, Liu F-T, Isseroff RR, Simon SI. Dynamics of Neutrophil Infiltration during Cutaneous Wound Healing and Infection Using Fluorescence Imaging. J Invest Dermatol 2008 Jan;128(7):1812-1820. PMC2617712

7. Daley JM, Brancato SK, Thomay AA, Reichner JS, Albina JE. The phenotype of murine wound macrophages. Journal of Leukocyte Biology 2010 Jan;87(1):59-67. PMC2801619

8. Egozi EI, Ferreira AM, Burns AL, Gamelli RL, Dipietro LA. Mast cells modulate the inflammatory but not the proliferative response in healing wounds. Wound Repair and Regeneration 2003 Jan;11(1):46-54.

9. Toulon A, Breton L, Taylor KR, Tenenhaus M, Bhavsar D, Lanigan C, Rudolph R, Jameson J, Havran WL. A role for human skin–resident T cells in wound healing. J Exp Med 2009 Apr;206(4):743-750. PMC2715110

10. Efron JE, Frankel HL, Lazarou SA, Wasserkrug HL, Barbul A. Wound healing and T-lymphocytes. Journal of Surgical Research 1990 May;48(5):460-463.

11. Jameson J, Ugarte K, Chen N, Yachi P, Fuchs E, Boismenu R, Havran WL. A role for skin gammadelta T cells in wound repair. Science 2002 Apr;296(5568):747-749.

12. Mills RE, Taylor KR, Podshivalova K, McKay DB, Jameson JM. Defects in Skin γδ T Cell Function Contribute to Delayed Wound Repair in Rapamycin-Treated Mice. The Journal of Immunology 2008;181(6):3974 -3983. PMC2547144

13. Brubaker AL, Schneider DF, Kovacs EJ. Neutrophils and natural killer T cells as negative regulators of wound healing. Expert Rev Dermatol 2011 Feb;6(1):5-8. PMC3063646

14. Cass DL, Bullard KM, Sylvester KG, Yang EY, Longaker MT, Adzick NS. Wound size and gestational age modulate scar formation in fetal wound repair,. Journal of Pediatric Surgery 1997 Mar;32(3):411-415.

15. Dovi JV, He L-K, DiPietro LA. Accelerated wound closure in neutrophil-depleted mice. Journal of Leukocyte Biology 2003 Apr;73(4):448 -455.

16. Martin P, D'Souza D, Martin J, Grose R, Cooper L, Maki R, McKercher SR. Wound Healing in the PU.1 Null Mouse--Tissue Repair Is Not Dependent on Inflammatory Cells. Current Biology 2003 Jul;13(13):1122-1128.

17. Goren I, Muller E, Schiefelbein D, Christen U, Pfeilschifter J, Muhl H, Frank S. Systemic Anti-TNF[alpha] Treatment Restores Diabetes-Impaired Skin Repair in ob/ob Mice by Inactivation of Macrophages. J Invest Dermatol 2007 Apr;127(9):2259-2267.

18. Ritis K, Doumas M, Mastellos D, Micheli A, Giaglis S, Magotti P, Rafail S, Kartalis G, Sideras P, Lambris JD. A novel C5a receptor-tissue factor cross-talk in neutrophils links innate immunity to coagulation pathways. J. Immunol 2006 Oct;177(7):4794-4802.

19. Jalili A, Shirvaikar N, Marquez-Curtis L, Qiu Y, Korol C, Lee H, Turner AR, Ratajczak MZ, Janowska-Wieczorek A. Fifth complement cascade protein (C5) cleavage fragments disrupt the SDF-1/CXCR4 axis: further evidence that innate immunity orchestrates the mobilization of hematopoietic stem/progenitor cells. Exp. Hematol 2010 Apr;38(4):321-332. PMC2884222

20. Hasty LA, Brockman WW, Lambris JD, Lyttle CR. Hormonal regulation of complement factor B in human endometrium. Am. J. Reprod. Immunol 1993 Oct;30(2-3):63-67.[cited 2011 May 20]

21. Tsonis PA, Lambris JD, Del Rio-Tsonis K. Adv. Exp. Med. Biol 2006;586:63-70.

22. Cole DS, Hughes TR, Gasque P, Morgan BP. Complement regulator loss on apoptotic neuronal cells causes increased complement activation and promotes both phagocytosis and cell lysis. Mol. Immunol 2006 May;43(12):1953-1964.

23. Alexander JJ, Anderson AJ, Barnum SR, Stevens B, Tenner AJ. The complement cascade: yin-yang in neuroinflammation--neuro-protection and -degeneration. J. Neurochem 2008 Dec;107(5):1169-1187.

24. Kimura Y, Miwa T, Zhou L, Song W-C. Activator-specific requirement of properdin in the initiation and amplification of the alternative pathway complement. Blood 2008 Jan;111(2):732-740. PMC2200840

25. Spitzer D, Mitchell LM, Atkinson JP, Hourcade DE. Properdin can initiate complement activation by binding specific target surfaces and providing a platform for de novo convertase assembly. J. Immunol 2007 Aug;179(4):2600-2608.

26. Dunkelberger JR, Song W-C. Complement and its role in innate and adaptive immune responses. Cell Res 2009 Dec;20(1):34-50.

27. Harboe M, Mollnes TE. The alternative complement pathway revisited. J. Cell. Mol. Med 2008 Aug;12(4):1074-1084.

28. Gullstrand B, Mårtensson U, Sturfelt G, Bengtsson AA, Truedsson L. Complement classical pathway components are all important in clearance of apoptotic and secondary necrotic cells. Clin. Exp. Immunol 2009 May;156(2):303-311. PMC2759479

29. Zhang X, Kimura Y, Fang C, Zhou L, Sfyroera G, Lambris JD, Wetsel RA, Miwa T, Song W-C. Regulation of Toll-like receptor-mediated inflammatory response by complement in vivo. Blood 2007 Jul;110(1):228-236. PMC1896115.

30. Weaver DJ, Reis ES, Pandey MK, Köhl G, Harris N, Gerard C, Köhl J. C5a receptor-deficient dendritic cells promote induction of Treg and Th17 cells. Eur. J. Immunol. 2010 Mar;40(3):710-721. PMC3040298

31. Marie JC, Astier AL, Rivailler P, Rabourdin-Combe C, Wild TF, Horvat B. Linking innate and acquired immunity: Divergent role of CD46 cytoplasmic domains in T cell-induced inflammation. Nature Immunology 2002;3(7):659-666.

32. Cardone J, Le Friec G, Vantourout P, Roberts A, Fuchs A, Jackson I, Suddason T, Lord G, Atkinson JP, Cope A, Hayday A, Kemper C. Complement regulator CD46 temporally regulates cytokine production by conventional and unconventional T cells. Nat Immunol 2010 print;11(9):862-871.

33. Kemper C, Chan AC, Green JM, Brett KA, Murphy KM, Atkinson JP. Activation of human CD4+ cells with CD3 and CD46 induces a T-regulatory cell 1 phenotype. Nature 2003 Jan;421(6921):388-392.

34. Ward PA. Role of C5 activation products in sepsis. ScientificWorldJournal 2010;10:2395-2402.

35. Teh BK, Yeo JG, Chern LM, Lu J. C1q regulation of dendritic cell development from monocytes with distinct cytokine production and T cell stimulation. Mol Immunol 2011 May;48(9-10):1128-38.

36. Fang C, Zhang X, Miwa T, Song W-C. Complement promotes the development of inflammatory T-helper 17 cells through synergistic interaction with Toll-like receptor signaling and interleukin-6 production. Blood 2009 Jul;114(5):1005-1015. PMC2721782

37. Han G, Geng S, Li Y, Chen G, Wang R, Li X, Ma Y, Shen B, Li Y. γδT-cell function in sepsis is modulated by C5a receptor signalling. Immunology 2011 Jul;133(3):340-349.

38. Pasch MC, Van Den Bosch NH, Daha MR, Bos JD, Asghar SS. Synthesis of complement components C3 and factor B in human keratinocytes is differentially regulated by cytokines. J. Invest. Dermatol 2000 Jan;114(1):78-82.

39. Purwar R, Bäumer W, Niebuhr M, Tschernig T, Kietzmann M, Werfel T. A protective role of complement component 3 in T cell-mediated skin inflammation. Exp Dermatol 2011 Sep;20(9):709-14; Epub 2011 May 16.

40. Kambas K, Chrysanthopoulou A, Kourtzelis I, Skordala M, Mitroulis I, Rafail S, Vradelis S, Sigalas I, Wu Y-Q, Speletas M, Kolios G, Ritis K. Endothelin-1 Signaling Promotes Fibrosis In Vitro in a Bronchopulmonary Dysplasia Model by Activating the Extrinsic Coagulation Cascade . J Immunol 2011 Jun 1;186(11):6568-75.

The present invention is not limited to the embodiments described and exemplified above, but is capable of variation and modification within the scope of the appended claims.

What is Claimed:

1. A method for promoting wound healing in an individual, the method comprising:

a) identifying an individual who has been wounded, suffers a chronic wound, or will be wounded; and

b) administering to the individual a therapeutically effective amount of a complement inhibitor to the individual, wherein the complement inhibitor reduces or prevents complement activation, thereby promoting healing of the wound.

2. The method of claim 1, wherein the complement inhibitor comprises one or more of a C3 inhibitor, a C3aR inhibitor, a C5a inhibitor, a C5aR inhibitor, a factor D inhibitor, a factor B inhibitor, a C4 inhibitor, a C1q inhibitor, or any combination thereof.

3. The method of claim 2, wherein the complement inhibitor is a C3 inhibitor.

4. The method of claim 3, wherein the C3 inhibitor is compstatin, a compstatin analog, a compstatin peptidomimetic, a compstatin derivative, or any combinations thereof.

5. The method of claim 4 wherein the C3 inhibitor comprises SEQ ID NO:1, SEQ ID NO.:2, SEQ ID NO:3 or SEQ ID NO:4.

6. The method of claim 2, wherein the complement inhibitor is a C5a inhibitor or a C5aR inhibitor.

7. The method of claim 6, wherein the C5a inhibitor or C5aR inhibitor is acetyl-Phe-[Orn-Pro-D-cyclohexylalanine-Trp-Arg] (PMX-53), PMX-53 analogs, neutrazumab, TNX-558, eculizumab, pexelizumab or ARC1905, or any combination thereof.

8. The method of claim 2, wherein the complement inhibitor is a C4 inhibitor.

9. The method of claim 1 wherein the individual is human.

10. The method of claim 1, wherein the complement inhibitor is administered systemically.

11. The method of claim 1, wherein the complement inhibitor is administered topically or locally.

12. The method of claim 1, wherein the complement inhibitor is administered together or concurrently with, or sequentially before or after, at least one other treatment for the wound.

13. A pharmaceutical composition for promoting wound healing in an individual, the pharmaceutical composition comprising one or more complement inhibitors and at least one other agent for treating the wound, in a pharmaceutically acceptable medium.

14. The composition of claim 13, wherein the complement inhibitor comprises one or more of a C3 inhibitor, a C3aR inhibitor, a C5a inhibitor, a C5aR inhibitor, a factor D inhibitor, a factor B inhibitor, a C4 inhibitor, a C1q inhibitor, or any combination thereof.

15. The composition of claim 14, wherein the complement inhibitor is a C3 inhibitor.

16. The composition of claim 15, wherein the C3 inhibitor is compstatin, a compstatin analog, a compstatin peptidomimetic, a compstatin derivative, or any combinations thereof.

17. The composition of claim 16, wherein the C3 inhibitor comprises SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 or SEQ ID NO: 4.

18. The composition of claim 14, wherein the complement inhibitor is a C5a inhibitor or a C5aR inhibitor.

19. The composition of claim 18, wherein the C5a inhibitor or C5aR inhibitor is acetyl-Phe-[Orn-Pro-D-cyclohexylalanine-Trp-Arg] (PMX-53), PMX-53 analogs, neutrazumab, TNX-558, eculizumab, pexelizumab or ARC1905, or any combination thereof.

20. The composition of claim 14, wherein the complement inhibitor is a C4 inhibitor.

21. The composition of claim 13, formulated for systemic administration.

22. The composition of claim 13, formulated for topical or local administration.

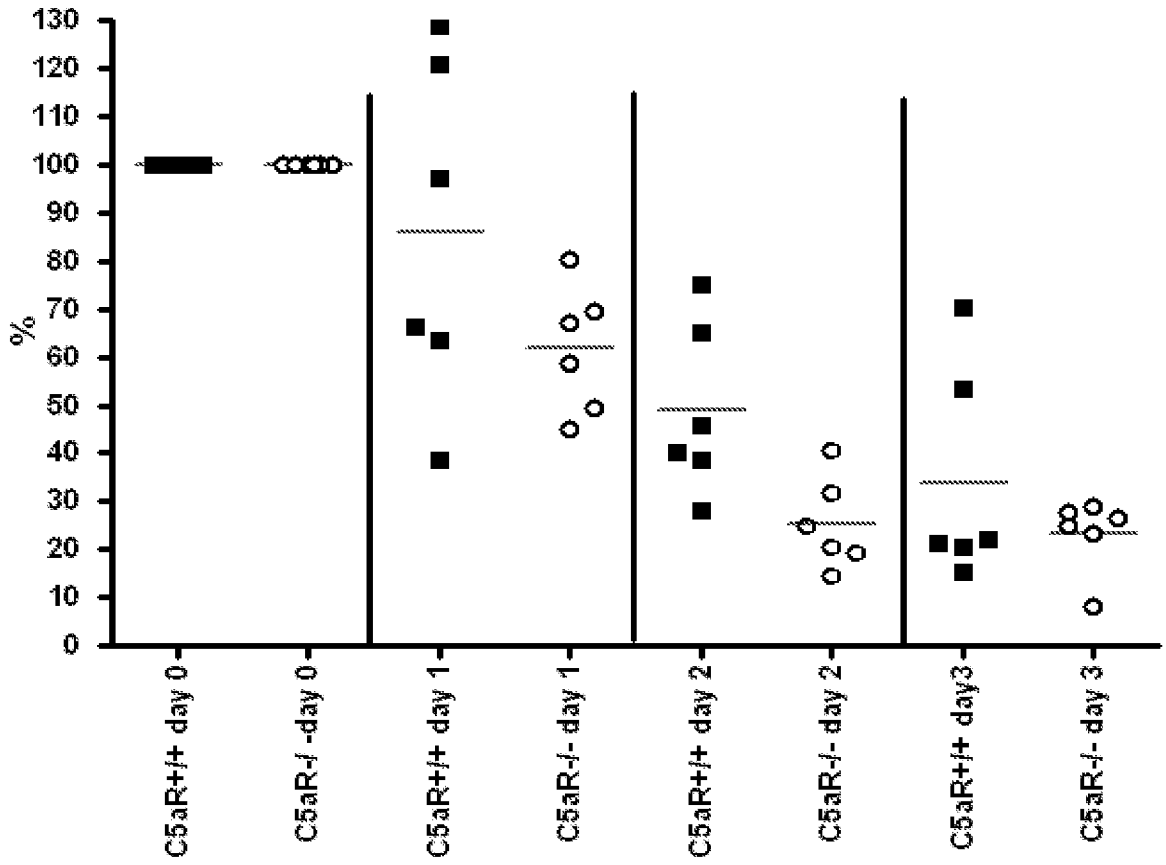
130
120
110
100
90
80
70
60
50
40
30
20
10
0
%
C5aR+/+ day 0
C5aR-/- day 0
C5aR+/+ day 1
C5aR-/- day 1
C5aR+/+ day 2
C5aR-/- day 2
C5aR+/+ day3
C5aR-/- day 3

Fig. 1

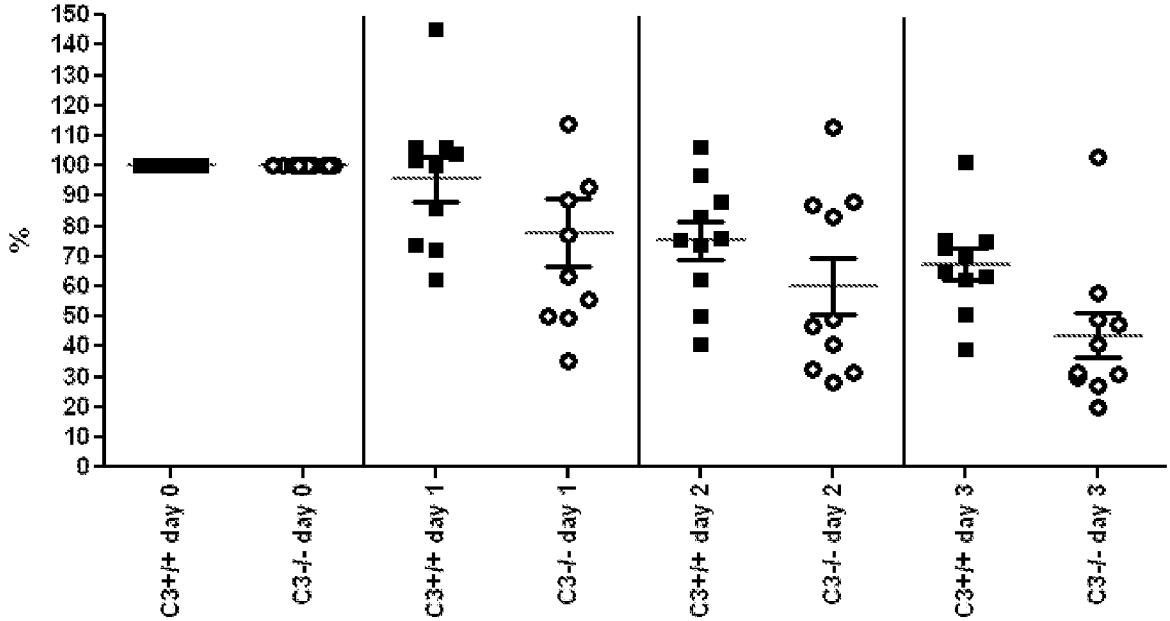
%
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
C3+/+ day 0
C3-/- day 0
C3+/+ day 1
C3-/- day 1
C3+/+ day 2
C3-/- day 2
C3+/+ day 3
C3-/- day 3

Fig. 2

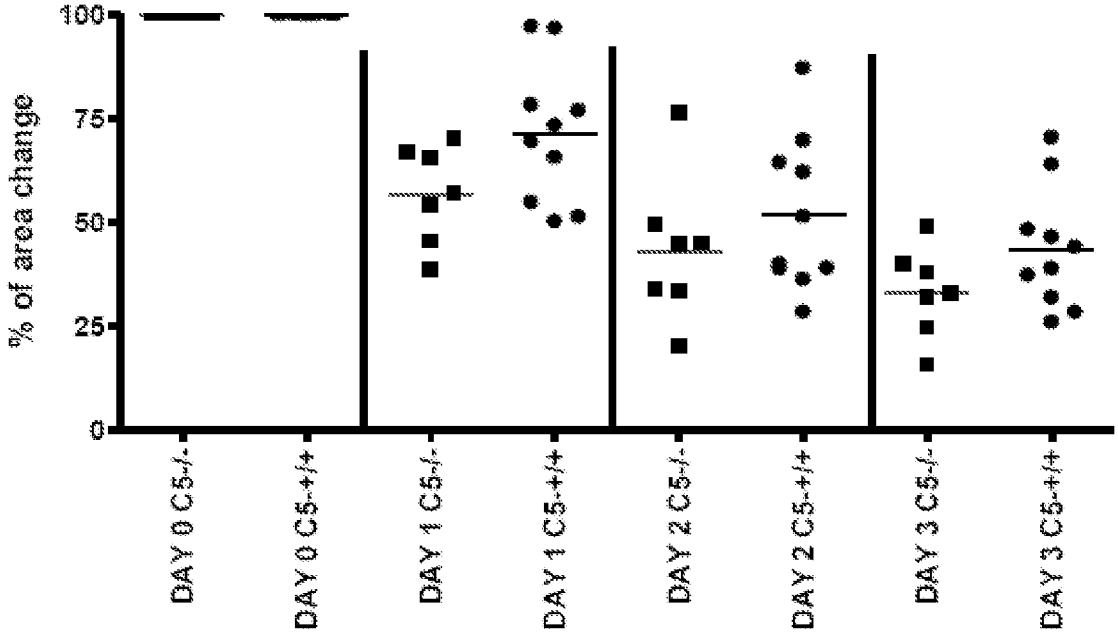
% of area change
100
75
50
25
0
DAY 0 C5-/-
DAY 0 C5-+/+
DAY 1 C5-/-
DAY 1 C5-+/+
DAY 2 C5-/-
DAY 2 C5-+/+
DAY 3 C5-/-
DAY 3 C5-+/+

Fig. 3

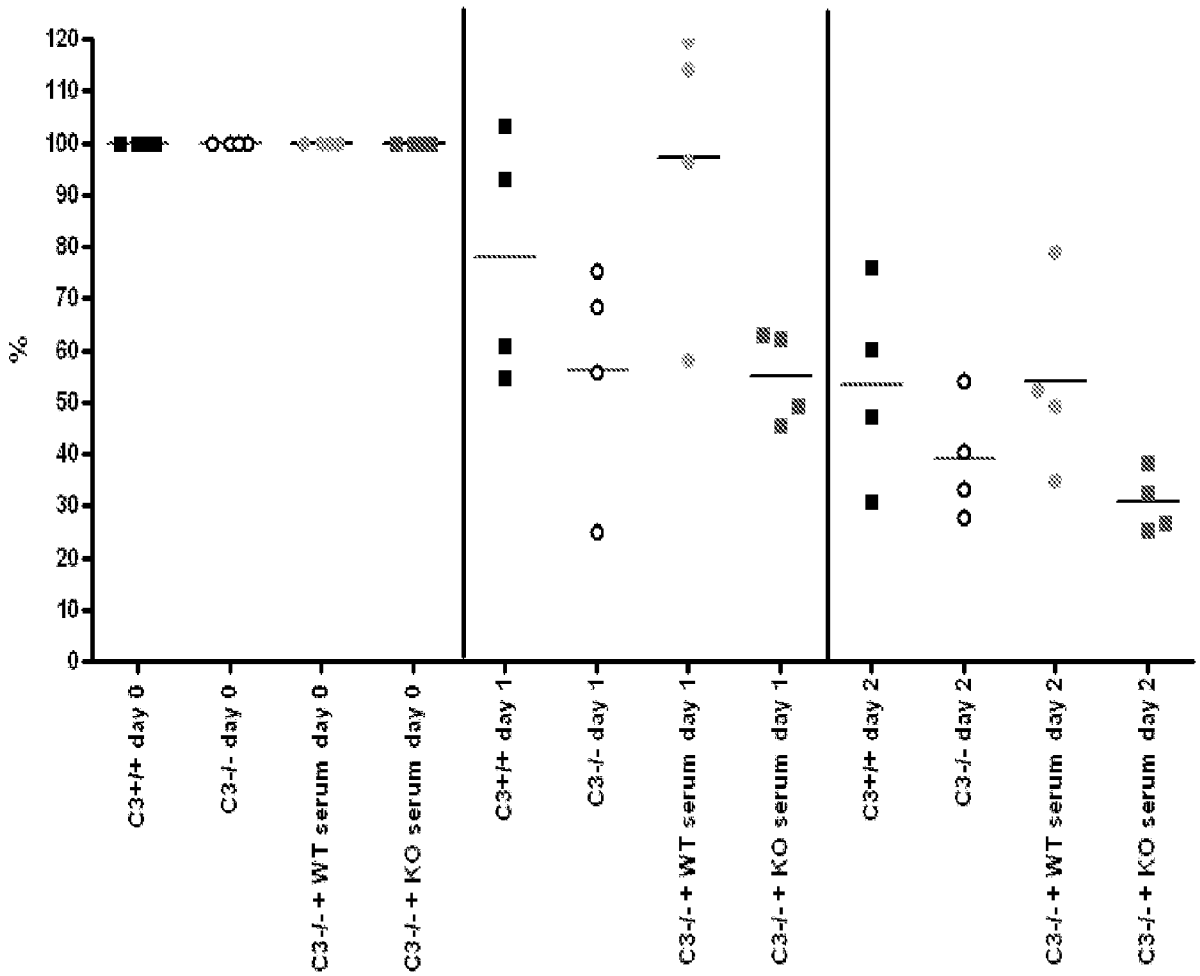
%
120
110
100
90
80
70
60
50
40
30
20
10
0
C3+/+ day 0
C3-/- day 0
C3-/- + WT serum day 0
C3-/- + KO serum day 0
C3+/+ day 1
C3-/- day 1
C3-/- + WT serum day 1
C3-/- + KO serum day 1
C3+/+ day 2
C3-/- day 2
C3-/- + WT serum day 2
C3-/- + KO serum day 2

Fig. 4

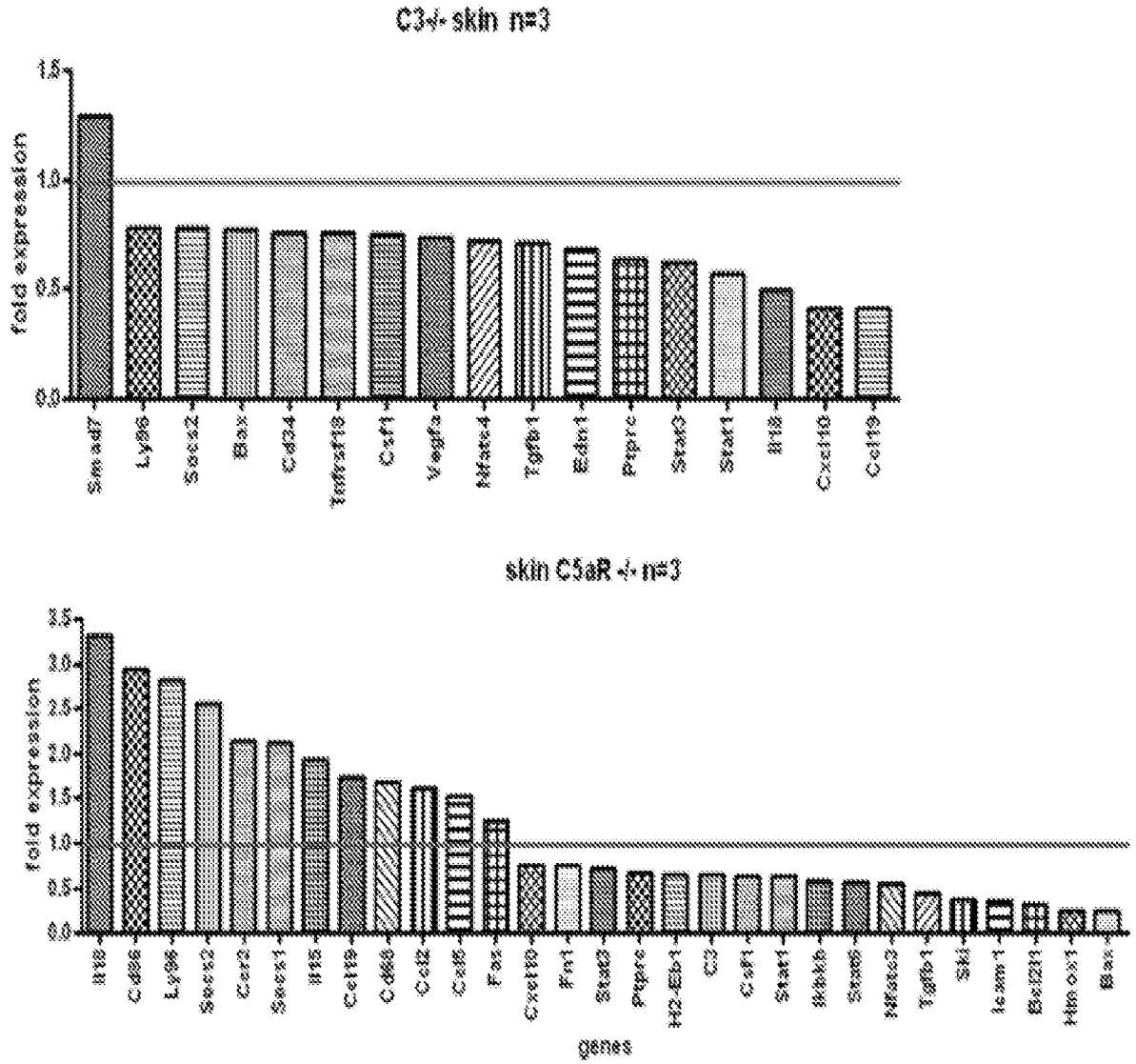
C3-/- skin n=3
fold expression
Smad7
Ly86
Socs2
Bax
Cd34
Tnfrsf19
Csf1
Vegfa
Mfsd2a
Tgfb1
Edn1
Pparc
Stat3
Start1
Il18
Cxcl10
Ccl19
skin C5aR -/- n=3
fold expression
Il18
Cd86
Ly86
Socs2
Car2
Socs1
Il1rl
Ccl19
Cd68
Cd2
Cxcl2
Fas
Cxcl10
Fn1
Stat3
Pparc
H2-Eb1
C3
Csf1
Start1
Ikbkb
Smad6
Mfsd2a
Tgfb1
Ski
Icosl
Bcl2l11
Hmox1
Bax
genes

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/042163

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/10 A61K38/12 A61K38/17 A61K31/00 A61K39/395
A61P17/02
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2003/022838 A1 (SHEPPARD PAUL O [US] ET AL) 30 January 2003 (2003-01-30) see [0015], [0052]-[0064], claims 53, 67, examples 12-17	1,2, 9-14,21, 22
X	WO 2009/014633 A1 (UNIV PENNSYLVANIA [US]; DEMOCRITUS UNIVERSITY OF THRAC [GR]; LAMBRIS J) 29 January 2009 (2009-01-29)	13-22
Y	see Seq.1, claims 1-23, ex.4 and 6	1-22

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 November 2012	29/11/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Merckling-Ruiz, V

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCTOR L M ET AL: "Complement inhibitors selectively attenuate injury following administration of cobra venom factor to rats", INTERNATIONAL IMMUNOPHARMACOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 6, no. 8, 1 August 2006 (2006-08-01), pages 1224-1232, XP024976882, ISSN: 1567-5769, DOI: 10.1016/J.INTIMP.2006.03.002 [retrieved on 2006-08-01]	13-15, 18,19
Y	abstract -----	1-22
Y	CLARK J DAVID ET AL: "Blockade of the complement C5a receptor reduces incisional allodynia, edema, and cytokine expression", ANESTHESIOLOGY (HAGERSTOWN), vol. 104, no. 6, June 2006 (2006-06), pages 1274-1282, XP002686652, ISSN: 0003-3022 abstract -----	1-22
Y	EHRNTHALLER CHRISTIAN ET AL: "New Insights of an Old Defense System: Structure, Function, and Clinical Relevance of the Complement System", MOLECULAR MEDICINE (BALTIMORE), vol. 17, no. 3-4, March 2011 (2011-03), pages 317-329, XP002686653, ISSN: 1076-1551 the whole document -----	1-22

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/042163

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2003022838 A1	30-01-2003	US 6544946 B1	08-04-2003
		US 2003022838 A1	30-01-2003
		US 2003144208 A1	31-07-2003
WO 2009014633 A1	29-01-2009	US 2011142837 A1	16-06-2011
		WO 2009014633 A1	29-01-2009