

TREATMENT OF INFLAMMATORY DISEASES WITH INHIBITORS OF C5a ACTIVITY**FIELD OF THE INVENTION**

5 The present invention relates to inhibitors of C5a activity and their use in the treatment of cutaneous, neutrophilic, inflammatory diseases in a subject.

BACKGROUND OF THE INVENTION**Target C5a in Inflammation**

10 C5a is a 74 amino acid spanning split product of its “mother molecule” C5 and represents one endpoint of the complement activation cascade. It can be generated through activation of at least three well-described pathways (the alternative, the classical and the MBL pathway). All pathways merge at the level of C3, forming the C5- or alternative C5 convertase leading to cleavage of C5 into C5a and C5b. The latter binds with C6, C7, C8 and multiple C9 molecules ultimately leading to formation of pores in e.g. bacterial membranes (terminal
15 Membrane Attack Complex = MAC). C5a is generated when the complement system is activated in settings of inflammation and other immunological and inflammatory disorders / diseases.

Among the complement activation products, C5a is one of the most potent inflammatory peptides, with a broad spectrum of functions (Guo and Ward, 2005). C5a exerts its effects
20 through the high-affinity C5a receptors (C5aR and C5L2) (Ward, 2009). C5aR belongs to the rhodopsin family of G-protein-coupled receptors with seven transmembrane segments; C5L2 has a similar structure but appears not to be G-protein-coupled. It is currently believed that C5a exerts its biological functions primarily through C5a-C5aR interaction, as few biological responses have been found for C5a-C5L2 interaction. However, latest reports demonstrate
25 signaling also through C5L2 activation (Rittirsch and others, 2008).

C5aR is widely expressed on myeloid cells including neutrophils, eosinophils, basophils, and monocytes, and non-myeloid cells in many organs, especially in the lung and liver, indicative of the importance of C5a/C5aR signaling. Widespread up-regulation of C5aR expression occurs during the onset of sepsis, and blockade of C5a/C5aR interaction by anti-
30 C5a, or anti-C5aR antibodies, or C5aR antagonists renders highly protective effects in rodent models of sepsis (Czermak and others, 1999; Huber-Lang and others, 2001; Riedemann and others, 2002).

C5a has a variety of biological functions (Guo and Ward, 2005). C5a is a strong chemoattractant for neutrophils and also has chemotactic activity for monocytes and macrophages. C5a causes an oxidative burst (O_2 consumption) in neutrophils and enhances phagocytosis and release of granular enzymes. C5a has also been found to be a vasodilator. C5a has been shown to be involved in modulation of cytokine expression from various cell types and to enhance expression of adhesion molecule expression on neutrophils. High doses of C5a can lead to nonspecific chemotactic “desensitization” of neutrophils, thereby causing broad dysfunction. Many inflammatory diseases are attributable to the effects of C5a, including sepsis, acute lung injury, inflammatory bowel disease, rheumatoid arthritis and others. In the experimental setting of sepsis, exposure of neutrophils to C5a can lead to neutrophil dysfunction and paralysis of signaling pathways, leading to defective assembly of NADPH oxidase, paralysis of MAPK signaling cascades, a great depression of oxidative burst, phagocytosis and chemotaxis (Guo and others, 2006; Huber-Lang and others, 2002). Thymocytes apoptosis and delayed neutrophil apoptosis are two important pathogenic events for sepsis development, which are dependent on the presence of C5a. During experimental sepsis, C5a up-regulates $\beta 2$ -integrin expression on neutrophils to promote cell migration into organs, one of the major causes for multi-organ failure (MOF). It is also found that C5a is attributable to the activation of the coagulation pathway that occurs in experimental sepsis. C5a stimulates the synthesis and release from human leukocytes of pro-inflammatory cytokines such as $TNF-\alpha$, $IL-1\beta$, $IL-6$, $IL-8$, and macrophage migration inhibitory factor (MIF). Given that complement activation is an event occurring during the onset of acute inflammation, C5a may come into play before emergence of most of the inflammatory “cytokine storm”. It appears that C5a plays a key role in orchestrating and amplifying the performance of the cytokine network and the formation of systemic inflammatory response syndrome (SIRS).

In the immunological regulatory network tailing to the adaptive immunity, C5a affects the crosstalk between dendritic cells (DC) and $\delta\epsilon T$ cells, and this may result in a large production of inflammatory mediators such as $IL-17$ (Xu and others, 2010). An essential role for C5a has been established and defined in the generation of pathogenic $Th17$ responses in systemic lupus erythematosus (SLE) (Pawaria and others, 2014). In addition, it has been reported that C5a is a key regulator for Treg cells offering a powerful suppressive effect for Treg propagation and induction (Strainic and others, 2013). Given the fact that Treg and $TH17$ are the essential players in the autoimmune disease setting, inhibition of C5a signaling would be expected to significantly reduce overactive immune status in the autoimmune diseases.

IFX-1

IFX-1 is a chimaeric monoclonal IgG4 antibody which specifically binds to the soluble human complement split product C5a. IFX-1 is composed of 1328 amino acids and has an approximate molecular weight of 148,472 Daltons. The CDR and FR sequences of IFX-1 are disclosed in in Table 3 below.

IFX-1 is expressed in a mammalian CHO cell line as recombinant protein and finally formulated in a phosphate buffered saline solution for intravenous administration. The binding of this antibody to human C5a facilitates a highly effective blockade of C5a-induced biological effects by disabling C5a binding to and reacting with its corresponding cell-bound receptors.

Various nonclinical studies were conducted to assess pharmacological and toxicological aspects of IFX-1, which can be divided into *in vitro* / *ex vivo* tests and *in vivo* studies including GLP toxicology studies in cynomolgus monkey (using IFX-1). None of the conducted nonclinical tests and studies revealed any toxicological or safety concerns for IFX-1. Human Phase I trial indicated that safety laboratory parameters, vital signs and ECG parameters showed no clinically relevant time or dose-related changes.

In vitro analysis of IFX-1 demonstrates a strong binding capacity to soluble human C5a as well as a high blocking activity of C5a-induced biological effects such as lysozyme release from human neutrophils or CD11b up-regulation in neutrophils in human whole blood. One IFX-1 antibody reaches the capability of neutralizing the effects of 2 molecules C5a with close to 100% efficiency in experimental *in vitro* settings. Clinical trials with IFX-1 have been ongoing to test its clinical efficacy in several inflammatory diseases including septic organ dysfunction and complex cardiac surgery.

Neutrophils

Neutrophils, terminally differentiated cells with a short lifespan in circulation, are the most abundant leukocytes in the human body. As a first line of defense against invading microorganisms, neutrophils are characterized by their ability to act as phagocytic cells, release lytic enzymes from their granules and produce reactive oxygen species upon stimulation. In addition to microbial products, other stimuli such as immune complex can also induce the respiratory burst in neutrophils, leading to enhanced inflammation and the recruitment of inflammatory cells (Kaplan, 2013).

After infiltrating into inflamed tissues, neutrophils engage in many other cell types, such as macrophages, dendritic cells (DCs), natural killer cells, lymphocytes and mesenchymal stem cells, regulate innate and adaptive immune responses. For instance, neutrophils can modulate

DC maturation and the proliferation and polarization of T cells, and they can also directly prime antigen-specific T-helper type 1 and T-helper type 17 cells (Abi Abdallah and others, 2011). A variety of stimuli induce neutrophil degranulation, including C5a, formyl-methionyl-leucyl-phenylalanine (FMLP), lipopolysaccharide, platelet activating factor, and Tumor necrosis factor (TNF) (Kaplan, 2013). The contents released from degranulation and oxidative species together with cytokines and chemokines resulted from neutrophil activation are the primary inflammatory mediators that cause tissue damage, and this mechanism is believed to be attributable to many types of inflammatory tissue injury.

10 **Hidradenitis Suppurativa (HS)**

HS is a chronic devastating skin disorder affecting areas rich in apocrine glands, and it is considered as one of neutrophil-associated cutaneous inflammatory diseases. Nodules appear in the affected areas, and they progressively become swollen and rupture with the release of pus. This process occurs repeatedly leading to sinus tract formation and scars (Jemec, 2004). This disease course creates a frustrating situation for the patients but also for physicians. The point prevalence is reported to range between 1% and 4% (Jemec and others, 1996).

The exact pathophysiology of HS is not well defined. Smoking, dietary habits and genetic predisposition have all been linked with HS (Kurzen and others, 2008; Slade and others, 2003). The percentage of NK cells was increased and that of CD4-lymphocytes decreased compared to healthy controls probably implying the existence of an autoimmune predilection for the disorder. IL-1 β and IL-17 have been found to be upregulated in the lesion of HS, being associated with the activation of inflammasome (Lima and others, 2016). Hidradenitis suppurativa (HS) is presented with the high number of neutrophil infiltrates in the inflamed skin, especially in the late stage of disease (Lima and others, 2016; Marzano, 2016). Activated neutrophils could be an important effector cell type causing tissue damage through direct harmful effect or indirect regulatory effect toward other effect cells such as active T cells and TH17 in this disease setting.

A hypothesis for the implication of some autoimmune or autoinflammatory mechanism in the pathogenesis of HS has been created over the last years. The hypothesis is further reinforced by positive results from the administration of TNF antagonists in prospective, placebo-controlled studies, which result in the approval of Adalimumab (an antibody directed against tumor necrosis factor α) in patients with moderate to severe HS. One major, yet unanswered question is how neutrophils are recruited to the affected skin lesion and to what extent activated neutrophils would contribute to the disease development.

The wide range of possible pathogenic mechanisms suggested by different studies may imply that HS is associated with host mechanisms rather than exogenous factors. Taking into account of the paradox that both anti-infectious (antibiotics) and pro-infectious (anti-TNF, corticosteroids, immunosuppressive drugs) therapies may be helpful, HS may appear as an auto-inflammatory disease based on a defect in the hair follicle innate immunity (Revuz, 2009), which is supported by the fact that pro-inflammatory cytokines such as interleukin (IL)-1 β , and tumor necrosis factor- α (TNF- α) are markedly increased in lesional and perilesional skin (Wollina and others, 2013).

10 Neutrophilic Dermatoses

The neutrophilic dermatoses (ND) are a group of disorders characterized by skin lesions for which histologic examination reveals intense inflammatory infiltrates composed primarily of neutrophils with no evidence of infection. ND mainly include Sweet syndrome (SS), pyoderma gangrenosum (PG), subcorneal pustular dermatosis (SPD), other well-defined entities, and their atypical or transitional forms (Prat and others, 2014). Hidradenitis suppurativa (HS) has recently been assigned to the family of ND based on the high number of neutrophil infiltrates observed in the inflamed skin (Lima and others, 2016; Marzano, 2016).

Pyoderma gangrenosum (PG) and hidradenitis suppurativa (HS) are prototypic neutrophilic dermatoses that are regarded as autoinflammatory disease in origin with the hallmark of the accumulation of neutrophils in the skin (Braun-Falco and others, 2012; Marzano and others, 2014). Autoinflammatory Syndrome represents an emerging group of inflammatory conditions that are distinct from autoimmune, allergic, and infectious disorders. From a pathophysiological perspective, all the autoinflammatory syndromes such as PAPA (pyogenic arthritis, PG and acne), PASH (PG, acne and hidradenitis suppurativa) or PAPASH (pyogenic arthritis, acne, PG and hidradenitis suppurativa) share common mechanisms consisting of over-activation of the innate immune system and ‘sterile’ neutrophil-rich cutaneous inflammation (Cugno and others, 2017).

BADAS (bowel-associated dermatosis-arthritis syndrome) is characterized by fever, flu-like symptoms, arthritis and inflammatory skin involvement. The latter is characterized by lesions recalling different neutrophilic dermatoses such as papules and plaques (Sweet’s syndrome), pustules and ulcers (pyoderma gangrenosum) or nodules, abscesses or fistulae (hidradenitis suppurativa). In addition, acne and neutrophilic panniculitis can be associated. Patients usually experience a symmetrical, non-erosive polyarthritis that predominantly involves small joints (Cugno and others, 2018).

Synovitis, acne, pustulosis, hyperostosis and osteitis (SAPHO) syndrome was initially described in 1987. SAPHO syndrome is a rare condition, possibly to misdiagnosis. Although its pathogenesis is still elusive, there is increasing understanding that SAPHO shares similarities with other autoinflammatory diseases (Cugno and others, 2018).

5

Neutrophils and Autoimmune Diseases

Autoimmune diseases are defined by defective differentiation of self and non-self molecules, leading to inappropriate recognition of self molecules and tissues as foreign structures, and concomitant immune attack against host organs. The pathogenesis of autoimmune diseases can generally be divided into two phases, immunization phase and effector phase. Immunization phase is characterized by the emergence of autoreactive T-lymphocytes. Those T-cells then trigger a secondary response leading to tissue damaging phase by activating various other cell types (B-cells, cytotoxic T-cells, NK-cells, neutrophils, macrophages, osteoclasts, fibroblasts, etc.). The activation of those effector cells by the autoreactive T cells can be considered as the effector phase which can be mediated by multiple levels including autoantibody production, cytokine networks or direct cell-cell contacts (Nemeth and Mocsai, 2012).

The role of neutrophils in the pathophysiological development of autoimmune diseases has been limitedly defined, but increasingly appreciated. Neutrophils could participate in the multiple steps of the autoimmune disease process, including antigen presentation, regulation of the activity of other immune cell types, and direct tissue damage. Neutrophils can expose/release autoantigens when activated, or when dying by apoptosis, or during formation of neutrophil extracellular traps (NETs). They can also contribute to tissue deposition of autoantibodies or, as an effector cell type, they can induce tissue damage themselves. Accumulative studies have demonstrated that neutrophils play an active role in the development of autoimmune diseases, such as, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), bullous pemphigoid, epidermolysis bullosa acquisita, ANCA-associated vasculitis, familial Mediterranean fever, cryopyrin-associated periodic disorders (CAPS) and gout, etc. (Nemeth and Mocsai, 2012; Nemeth and others, 2016). As the skin being an easy target for immune responses, cutaneous inflammation is one of most frequent syndromes presented by these autoimmune diseases. However, rheumatoid neutrophilic dermatosis is a rare cutaneous manifestation in patients with severe rheumatoid arthritis. It mainly affects patients with severe seropositive rheumatoid arthritis, predominantly women (ration 2:1), but it has been observed also in seronegative rheumatoid arthritis (Cugno and others, 2018).

TECHNICAL PROBLEMS UNDERLYING THE PRESENT INVENTION

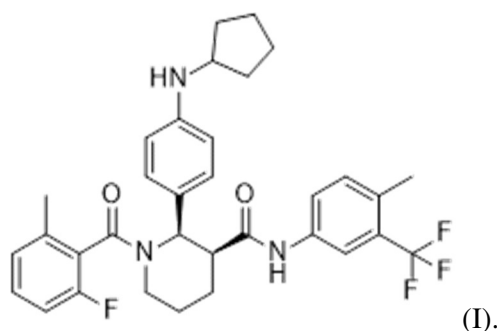
As explained above, there existed a need in the prior art for effective therapies for the treatment of neutrophilic dermatoses, such as Hidradenitis suppurativa (HS), and cutaneous neutrophilic autoimmune diseases.

5 The present inventors have now surprisingly found that molecules inhibiting C5a signaling, e.g. an anti-C5a antibody, are exceptionally well-suited for the treatment of Hidradenitis suppurativa. The present inventors have additionally studied the physiological mechanism leading to neutrophil activation and found out that C5a is the key driver of neutrophil activation.

10 Thus, the present inventors expect that inhibiting C5a activity will be a suitable therapy approach for the treatment of various neutrophilic disorders, especially cutaneous, neutrophilic, inflammatory diseases.

SUMMARY

15 In one aspect the present disclosure refers to the use of a compound in the manufacture of a medicament for treating a cutaneous, neutrophilic, inflammatory disease in a subject, wherein the compound is an inhibitor of C5a activity, and wherein the cutaneous, neutrophilic, inflammatory disease is selected from the group consisting of hidradenitis suppurativa (HS); PASH (PG, acne and hidradenitis suppurativa); PAPASH (pyogenic arthritis, acne, PG and hidradenitis suppurativa), wherein the inhibitor of C5a activity directly binds to a C5a receptor and inhibits the activity of the
20 C5a, wherein the inhibitor of C5a activity has a structure according to formula (I):



25 In one example, there is disclosed a method for the treatment of a cutaneous, neutrophilic, inflammatory disease in a subject, comprising the step of: administering to a subject in need thereof a therapeutic amount of a compound, wherein the compound is an inhibitor of C5a activity, and wherein the cutaneous, neutrophilic, inflammatory disease is selected from the group consisting of hidradenitis suppurativa (HS); Pyoderma gangrenosum (PG); PAPA (pyogenic arthritis, PG and acne); PASH (PG, acne and hidradenitis suppurativa); PAPASH (pyogenic arthritis, acne, PG and hidradenitis suppurativa); Sweet syndrome (SS); subcorneal pustular dermatosis (SPD); epidermolysis bullosa acquisita,

erythema elevatum diutinum (EED); neutrophilic panniculitis; bowel-associated dermatosis-arthritis syndrome (BADAS); SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis) syndrome; rheumatoid neutrophilic dermatosis; familial Mediterranean fever, cryopyrin-associated disorders, gout, and Schnitzler syndrome.

5 In a further example, there is disclosed a use of a compound for the preparation of a pharmaceutical composition for the treatment of a cutaneous, neutrophilic, inflammatory disease, wherein the compound is an inhibitor of C5a activity, and wherein the cutaneous, neutrophilic, inflammatory disease is selected from the group consisting of hidradenitis suppurativa (HS);
 10 Pyoderma gangrenosum (PG); PAPA (pyogenic arthritis, PG and acne); PASH (PG, acne and hidradenitis suppurativa); PAPASH (pyogenic arthritis, acne, PG and hidradenitis suppurativa); Sweet syndrome (SS); subcorneal pustular dermatosis (SPD); epidermolysis bullosa acquisita, erythema elevatum diutinum (EED); neutrophilic panniculitis; bowel-associated dermatosis-arthritis syndrome (BADAS); SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis) syndrome; rheumatoid neutrophilic dermatosis; familial Mediterranean fever, cryopyrin-
 15 associated disorders, gout, and Schnitzler syndrome.

This summary of the invention does not necessarily describe all features of the present invention. Other embodiments will become apparent from a review of the ensuing detailed description.

20

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Blocking activity of IFX-1 to recombinant human C5a (rhC5a)-induced CD11b upregulation on blood neutrophils. IFX-1-004 and IFX-1-012 represent two different production batches. Human whole blood was incubated with buffer, antibody alone, rhC5a alone,
 25 or combinations of different antibody concentration and rhC5a. After incubation, cells were stained with anti-mouse CD11b:FITC and CD11b MFI was analysed by flow cytometry. Results are presented as mean $\pm$ SD. The percentage of IFX-1 blocking activity of C5a-induced CD11b expression is marked (arrow). Statistical differences were calculated by One-Way-ANOVA, p values of $p < 0.05$ were statistically significant.

30 **Figure 2. Blocking activity of IFX-1 on endogenous C5a (eC5a)-driven CD11b upregulation on neutrophils.** Zymosan-activated human plasma (ZAP) was used as the source of eC5a. Whole blood was incubated with buffer, IFX-1 alone, ZAP alone, or combinations of IFX-1 and ZAP. After incubation, cells were stained with anti-mouse CD11b:FITC and analysed by flow cytometry. Results are presented as mean $\pm$ SD. The percentage of IFX-1

blocking activity of eC5a-induced CD11b expression is marked (arrow). Statistical differences were calculated by One-Way-ANOVA, p values of $p < 0.05$ were statistically significant.

Figure 3. Activation of blood neutrophils by zymosan and IFX-1 blocking activity.

Whole blood was incubated with HBSS, rhC5a and zymosan A alone, or combinations of different IFX-1 concentrations and rhC5a or zymosan A. After incubation, cells were stained with anti-mouse CD11b:FITC, and CD11b MFI was analysed by flow cytometry. Results are presented as mean $\pm$ SD. The percentage of IFX-1 blocking activity of C5a-induced CD11b expression is marked (arrow). Statistical differences were calculated by One-Way-ANOVA, p values of $p < 0.05$ were statistically significant.

Figure 4. IFX-1 inhibits zymosan-induced generation of IL-8 in human whole blood.

IL-8 concentrations were obtained by ELISA after incubation of human whole blood with different concentrations of zymosan A (as indicated on the x-axis) in the presence (empty circles) or absence (filled circles) of IFX-1. Results were presented as mean $\pm$ SD.

Figure 5. Concentrations of C3a (A), C5a (B) and C5b-9 (C) in the plasma of 14

healthy controls and of 54 patients with hidradenitis suppurativa (HS). Circles denote outliers and asterisks denote extremes. P values symbolize significant differences between patients and controls.

Figure 6. Effect of HS plasma on blood neutrophil activation and the potential role of C5a.

HS plasma samples were incubated with human whole blood in the presence and absence of IFX-1, and CD11b expression on blood neutrophils was determined by flow cytometric analysis. C5a levels in the control and HS samples were labeled in the embedded table.

Figure 7. HiSCR response post-IFX-1 treatment in HS patients.

HiSCR responder is defined as a $\geq 50\%$ reduction in inflammatory lesion count (abscesses + inflammatory nodules), and no increase in abscesses or draining fistulas when compared with baseline.

Figure 8. Blockade of C5a-induced CD11b upregulation via different anti-C5aR antibodies.

Whole blood as source of neutrophils was incubated with (spiked-) plasma samples in the absence or presence of respective anti-C5aR antibodies. The blocking activity of each inhibitor was indicated as percentage on the corresponding sample.

Figure 9. Blockade of C5a-induced CD11b upregulation via C5aR antagonist PMX-53.

Whole blood as source of neutrophils was incubated with (spiked-) plasma samples in the absence or presence of low-concentrated (A) or over-concentrated (B) C5aR antagonist PMX-53. The blocking activities of PMX-53 were indicated as percentage on the corresponding samples.

Figure 10. Blockade of C5a-induced CD11b upregulation via C5a blocking antibody IFX-1. Whole blood as source of neutrophils was incubated with (spiked-) plasma samples in the absence or presence of IFX-1. The blocking activities of IFX-1 were indicated as percentage on the corresponding samples.

Figure 11. Blockade of C5a-induced CD11b upregulation via C5aR inhibitor Avacopan. Whole blood as source of neutrophils was incubated with (spiked-) plasma samples in the absence or presence of Avacopan. The blocking activities of Avacopan were indicated as percentage on the corresponding samples.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and Kölbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Several documents (for example: patents, patent applications, scientific publications, manufacturer's specifications, instructions, GenBank Accession Number sequence submissions etc.) are cited throughout the text of this specification. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. Some of the documents cited herein are characterized as being "*incorporated by reference*". In the event of a conflict between the definitions or teachings of such incorporated references and definitions or teachings recited in the present specification, the text of the present specification takes precedence.

Sequences: All sequences referred to herein are disclosed in the attached sequence listing that, with its whole content and disclosure, is a part of this specification.

In the context of the present invention, C5a particularly refers to human C5a. Human C5a is a 74 amino acid peptide with the following amino acid sequence:

5 TLQKKIEEIA AKYKHSVVKK CCYDGACVNN DETCEQRAAR ISLGPRCIKA
 FTECCVVASQ LRANISHKDM QLGR (SEQ ID NO: 1).

The amino acid sequence of human C5 can be found under the accession number UniProtKB P01031 (CO5_HUMAN).

10 As used herein, the term “inhibitor of C5a activity” refers to any compound that in any way reduces the activity of C5a. This activity reduction can be achieved by directly or indirectly lowering the concentration of C5a, or by reducing the activity of C5a, or by preventing C5a from exerting its effects on one or more of its receptors (e.g. on C5aR or C5L2), or by reducing the concentration or activity of one or more receptors of C5a.

15 In the context of the present invention, the expression “C5a receptor” refers to any potential C5a binding ligand on the cell surface, especially to any receptor protein to which C5a may bind and elicit a reaction on said receptor (e.g. activation or inhibition of the receptor). The term “C5a receptor” particularly encompasses the two receptors C5aR and C5L2. Alternative names for C5aR are C5aR1 and CD88. An alternative name for C5L2 is C5aR2.

20 Certain embodiments of the present invention refer to an inhibitor of C5a that interferes with a C5a receptor (e.g. by binding to a C5a receptor, or by blocking expression of a C5a receptor). In these contexts, the term “a C5a receptor” can refer to (i) C5aR or to (ii) C5L2 or to (iii) both C5aR and C5L2. This means that some inhibitors of C5a interfere with only one of the C5a receptors (i.e. either C5aR or C5L2), while other inhibitors of C5a interfere with both C5a receptors (i.e. both C5aR and C5L2).

25 In the context of the present invention, the expression “protein ligand” refers to any molecule composed of amino acids linked by peptide bonds, irrespective of the total size of the molecule, and that is capable of specifically binding to another molecule. Accordingly, the expression “protein ligand” comprises oligopeptides (≤ 100 amino acids) and polypeptides (> 100 amino acids). The expression “protein ligand” also comprises cyclic peptides, 30 irrespective of their size. The expression “protein ligand” particularly encompasses antibodies, antigen-binding fragments of antibodies, antibody-like proteins, and peptidomimetics.

As used herein, a first compound (e.g. a protein ligand or nucleic acid aptamer) is considered to “bind” to a second compound (e.g. a target protein), if it has a dissociation constant K_d to said second compound of 1 mM or less, preferably 100 μ M or less, preferably

50 μM or less, preferably 30 μM or less, preferably 20 μM or less, preferably 10 μM or less, preferably 5 μM or less, more preferably 1 μM or less, more preferably 900 nM or less, more preferably 800 nM or less, more preferably 700 nM or less, more preferably 600 nM or less, more preferably 500 nM or less, more preferably 400 nM or less, more preferably 300 nM or less, more preferably 200 nM or less, even more preferably 100 nM or less, even more preferably 90 nM or less, even more preferably 80 nM or less, even more preferably 70 nM or less, even more preferably 60 nM or less, even more preferably 50 nM or less, even more preferably 40 nM or less, even more preferably 30 nM or less, even more preferably 20 nM or less, and even more preferably 10 nM or less.

The term “binding” according to the invention preferably relates to a specific binding. “Specific binding” means that a compound (e.g. a protein ligand or nucleic acid aptamer) binds stronger to a target such as an epitope for which it is specific compared to the binding to another target. A compound binds stronger to a first target compared to a second target, if it binds to the first target with a dissociation constant (K_d) which is lower than the dissociation constant for the second target. Preferably the dissociation constant (K_d) for the target to which the compound binds specifically is more than 10-fold, preferably more than 20-fold, more preferably more than 50-fold, even more preferably more than 100-fold, 200-fold, 500-fold or 1000-fold lower than the dissociation constant (K_d) for the target to which the compound does not bind specifically.

As used herein, the term “ K_d ” (usually measured in “mol/L”, sometimes abbreviated as “M”) is intended to refer to the dissociation equilibrium constant of the particular interaction between a compound (e.g. a protein ligand) and a target molecule.

Methods for determining binding affinities of compounds, i.e. for determining the dissociation constant K_d , are known to a person of ordinary skill in the art and can be selected for instance from the following methods known in the art: Surface Plasmon Resonance (SPR) based technology, Bio-layer interferometry (BLI), enzyme-linked immunosorbent assay (ELISA), flow cytometry, isothermal titration calorimetry (ITC), analytical ultracentrifugation, radioimmunoassay (RIA or IRMA) and enhanced chemiluminescence (ECL). Typically, the dissociation constant K_d is determined at 20°C, 25°C, 30°C, or 37°C. If not specifically indicated otherwise, the K_d values recited herein are determined at 20 °C by ELISA.

An “epitope”, also known as antigenic determinant, is the part of a macromolecule that is recognized by the immune system, specifically by antibodies, B cells, or T cells. As used herein, an “epitope” is the part of a macromolecule capable of binding to a compound (e.g. an antibody or antigen-binding fragment thereof) as described herein. In this context, the term

“binding” preferably relates to a specific binding. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes can be distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

A “paratope” is the part of an antibody that binds to the epitope. In the context of the present invention, a “paratope” is the part of a compound (e.g. a protein ligand) as described herein that binds to the epitope.

The term “antibody” typically refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen-binding portion thereof. The term “antibody” also includes all recombinant forms of antibodies, in particular of the antibodies described herein, e.g. antibodies expressed in prokaryotes, unglycosylated antibodies, antibodies expressed in eukaryotes (e.g. CHO cells), glycosylated antibodies, and any antigen-binding antibody fragments and derivatives as described below.

Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H or V_H) and a heavy chain constant region. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L or V_L) and a light chain constant region. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

The term “antigen-binding fragment” of an antibody (or simply “binding portion”), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term “antigen-binding portion” of an antibody include (i) Fab fragments, monovalent fragments consisting of the V_L, V_H, C_L and C_H domains; (ii) F(ab')₂ fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) Fd fragments consisting of the V_H and C_H domains; (iv) Fv fragments consisting of the V_L and V_H domains of a single arm of an antibody, (v) dAb fragments (Ward et al., (1989) Nature

341: 544-546), which consist of a V_H domain; (vi) isolated complementarity determining regions (CDR), and (vii) combinations of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) *Science* 242: 423-426; and Huston et al. (1988) *Proc. Natl. Acad. Sci. USA* 85: 5879-5883). Such single chain antibodies are also intended to be encompassed within the term “antigen-binding fragment” of an antibody. A further example is a binding-domain immunoglobulin fusion protein comprising (i) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, (ii) an immunoglobulin heavy chain CH₂ constant region fused to the hinge region, and (iii) an immunoglobulin heavy chain CH₃ constant region fused to the CH₂ constant region. The binding domain polypeptide can be a heavy chain variable region or a light chain variable region. The binding-domain immunoglobulin fusion proteins are further disclosed in US 2003/0118592 and US 2003/0133939. These antibody fragments are obtained using conventional techniques known to those skilled in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Further examples of “antigen-binding fragments” are so-called microantibodies, which are derived from single CDRs. For example, Heap et al., 2005, describe a 17 amino acid residue microantibody derived from the heavy chain CDR3 of an antibody directed against the gp120 envelope glycoprotein of HIV-1 (Heap C.J. et al. (2005) *Analysis of a 17-amino acid residue, virus-neutralizing microantibody*. *J. Gen. Virol.* 86:1791-1800). Other examples include small antibody mimetics comprising two or more CDR regions that are fused to each other, preferably by cognate framework regions. Such a small antibody mimetic comprising V_H CDR1 and V_L CDR3 linked by the cognate V_H FR2 has been described by Qiu et al., 2007 (Qiu X.-Q. et al. (2007) *Small antibody mimetics comprising two complementary-determining regions and a framework region for tumor targeting*. *Nature biotechnology* 25(8):921-929).

Thus, the term “antibody or antigen-binding fragment thereof”, as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e. molecules that contain an antigen-binding site that immunospecifically binds an antigen. Also comprised are immunoglobulin-like proteins that are selected through techniques including, for example, phage display to specifically bind to a target molecule or target epitope. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD,

IgA and IgY), class (e.g., IgG1, IgG2, preferably IgG2a and IgG2b, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

Antibodies and antigen-binding fragments thereof usable in the invention may be from any animal origin including birds and mammals. Preferably, the antibodies or fragments are from human, chimpanzee, rodent (e.g. mouse, rat, guinea pig, or rabbit), chicken, turkey, pig, sheep, goat, camel, cow, horse, donkey, cat, or dog origin. It is particularly preferred that the antibodies are of human or murine origin. Antibodies of the invention also include chimeric molecules in which an antibody constant region derived from one species, preferably human, is combined with the antigen-binding site derived from another species, e.g. mouse. Moreover, antibodies of the invention include humanized molecules in which the antigen-binding sites of an antibody derived from a non-human species (e.g. from mouse) are combined with constant and framework regions of human origin.

As exemplified herein, antibodies of the invention can be obtained directly from hybridomas which express the antibody, or can be cloned and recombinantly expressed in a host cell (e.g., a CHO cell, or a lymphocytic cell). Further examples of host cells are microorganisms, such as *E. coli*, and fungi, such as yeast. Alternatively, they can be produced recombinantly in a transgenic non-human animal or plant.

The term “chimeric antibody” refers to those antibodies wherein one portion of each of the amino acid sequences of heavy and light chains is homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular class, while the remaining segment of the chain is homologous to corresponding sequences in another species or class. Typically the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals, while the constant portions are homologous to sequences of antibodies derived from another. One clear advantage to such chimeric forms is that the variable region can conveniently be derived from presently known sources using readily available B-cells or hybridomas from non-human host organisms in combination with constant regions derived from, for example, human cell preparations. While the variable region has the advantage of ease of preparation and the specificity is not affected by the source, the constant region being human is less likely to elicit an immune response from a human subject when the antibodies are injected than would the constant region from a non-human source. However, the definition is not limited to this particular example.

The term “humanized antibody” refers to a molecule having an antigen-binding site that is substantially derived from an immunoglobulin from a non-human species, wherein the remaining immunoglobulin structure of the molecule is based upon the structure and/or

sequence of a human immunoglobulin. The antigen-binding site may either comprise complete variable domains fused onto constant domains or only the complementarity determining regions (CDR) grafted onto appropriate framework regions in the variable domains. Antigen-binding sites may be wild-type or modified by one or more amino acid substitutions, e.g. modified to resemble human immunoglobulins more closely. Some forms of humanized antibodies preserve all CDR sequences (for example a humanized mouse antibody which contains all six CDRs from the mouse antibody). Other forms have one or more CDRs which are altered with respect to the original antibody.

Different methods for humanizing antibodies are known to the skilled person, as reviewed by Almagro & Fransson, 2008, *Frontiers in Bioscience*, 13:1619-1633, the content of which is herein incorporated by reference in its entirety. The review article by Almagro & Fransson is briefly summarized in US 2012/0231008 A1 which is the national stage entry of international patent application WO 2011/063980 A1. The contents of US 2012/0231008 A1 and WO 2011/063980 A1 are herein incorporated by reference in their entirety.

As used herein, “human antibodies” include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). Human antibodies of the invention include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described for example in U.S. Patent No. 5,939,598 by Kucherlapati & Jakobovits.

The term “monoclonal antibody” as used herein refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody displays a single binding specificity and affinity for a particular epitope. In one embodiment, the monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a non-human animal, e.g. mouse, fused to an immortalized cell.

The term “recombinant antibody”, as used herein, includes all antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal with respect to the immunoglobulin genes or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody, e.g. from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial antibody library, and (d) antibodies prepared, expressed, created

or isolated by any other means that involve splicing of immunoglobulin gene sequences to other DNA sequences.

The term “transfectoma”, as used herein, includes recombinant eukaryotic host cells expressing an antibody, such as CHO cells, NS/0 cells, HEK293 cells, HEK293T cells, plant cells, or fungi, including yeast cells.

As used herein, a “heterologous antibody” is defined in relation to a transgenic organism producing such an antibody. This term refers to an antibody having an amino acid sequence or an encoding nucleic acid sequence corresponding to that found in an organism not consisting of the transgenic organism, and being generally derived from a species other than the transgenic organism.

As used herein, a “heterohybrid antibody” refers to an antibody having light and heavy chains of different organismal origins. For example, an antibody having a human heavy chain associated with a murine light chain is a heterohybrid antibody.

Thus, “antibodies and antigen-binding fragments thereof” suitable for use in the present invention include, but are not limited to, polyclonal, monoclonal, monovalent, bispecific, heteroconjugate, multispecific, recombinant, heterologous, heterohybrid, chimeric, humanized (in particular CDR-grafted), deimmunized, or human antibodies, Fab fragments, Fab' fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, Fd, Fv, disulfide-linked Fvs (dsFv), single chain antibodies (e.g. scFv), diabodies or tetrabodies (Holliger P. et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90(14), 6444-6448), nanobodies (also known as single domain antibodies), anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies described herein), and epitope-binding fragments of any of the above.

The antibodies described herein are preferably isolated. An “isolated antibody” as used herein, is intended to refer to an antibody which is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds to C5a is substantially free of antibodies that specifically bind antigens other than C5a). An isolated antibody that specifically binds to an epitope, isoform or variant of human C5a may, however, have cross-reactivity to other related antigens, e.g. from other species (e.g. C5a species homologs, such as rat C5a). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals. In one embodiment of the invention, a combination of “isolated” monoclonal antibodies relates to antibodies having different specificities and being combined in a well-defined composition.

The term “naturally occurring”, as used herein, as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence

that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring.

As used herein, the term “nucleic acid aptamer” refers to a nucleic acid molecule that has been engineered through repeated rounds of *in vitro selection* or SELEX (systematic evolution of ligands by exponential enrichment) to bind to a target molecule (for a review see: Brody E.N. and Gold L. (2000), *Aptamers as therapeutic and diagnostic agents*. J. Biotechnol. 74(1):5-13). The nucleic acid aptamer may be a DNA or RNA molecule. The aptamers may contain modifications, e.g. modified nucleotides such as 2'-fluorine-substituted pyrimidines, and/or may comprise one or more nucleotides with L-ribose units (or L-deoxyribose) instead of the standard D-ribose units (or D-deoxyribose units).

As used herein, the term “antibody-like protein” refers to a protein that has been engineered (e.g. by mutagenesis of loops) to specifically bind to a target molecule. Typically, such an antibody-like protein comprises at least one variable peptide loop attached at both ends to a protein scaffold. This double structural constraint greatly increases the binding affinity of the antibody-like protein to levels comparable to that of an antibody. The length of the variable peptide loop typically consists of 10 to 20 amino acids. The scaffold protein may be any protein having good solubility properties. Preferably, the scaffold protein is a small globular protein. Antibody-like proteins include without limitation affibodies, affilins, affimers, affitins, alphabodies, anticalins, avimers, DARPins (designed ankyrin repeat proteins), fynomers, Kunitz domain peptides, and monobodies (for review see: Binz H.K. et al. (2005) *Engineering novel binding proteins from nonimmunoglobulin domains*. Nat. Biotechnol. 23(10):1257-1268). Antibody-like proteins can be derived from large libraries of mutants, e.g. be panned from large phage display libraries and can be isolated in analogy to regular antibodies. Also, antibody-like binding proteins can be obtained by combinatorial mutagenesis of surface-exposed residues in globular proteins. Antibody-like proteins are sometimes referred to as “peptide aptamers” or as “antibody mimetics”.

As used herein, a “peptidomimetic” is a small protein-like chain designed to mimic a peptide. Peptidomimetics typically arise from modification of an existing peptide in order to alter the molecule's properties. For example, they may arise from modifications to change the molecule's stability or biological activity. This can have a role in the development of drug-like compounds from existing peptides. These modifications involve changes to the peptide that will not occur naturally (such as altered backbones and the incorporation of non-natural amino acids).

In the context of the present invention, the term “small molecule” refers to a molecule with a molecular weight of 2 kDa or less, preferably with a molecular weight of 1 kDa or less. The term “small molecule” particularly refers to molecules that are neither oligopeptides nor oligonucleotides.

5 In the context of the present invention, the general expression “wherein A competes with B for binding to C”, (e.g. in the expression “*wherein said antibody or antigen-binding fragment thereof competes with one of the antibodies indicated under (a) for binding to C5a*”) is used to define the binding properties of the compound listed in position A. Said compound A binds to C and compound B also binds to C but compound A and compound B cannot bind
10 to C at the same time; i.e. A and B bind to the same epitope (or at least to overlapping epitopes) on C. Such competition in binding can be determined by competitive ELISA or by Surface Plasmon Resonance (SPR) based technology or by any of the other techniques listed above in the context of the determination of binding affinities. If not explicitly stated otherwise, the competing binding properties of a compound are determined by ELISA at 20°C using equimolar
15 concentrations of the two competing compounds.

As used herein, a “cutaneous, neutrophilic, inflammatory disease” refers to any disease that is associated with an inflammation of the skin and with a neutrophilic infiltrate into the skin (e.g. into the epidermis) of an individual afflicted by said disease. The term “cutaneous, neutrophilic, inflammatory disease” particularly refers to hidradenitis suppurativa (HS);
20 Pyoderma gangrenosum (PG); PAPA (pyogenic arthritis, PG and acne); PASH (PG, acne and hidradenitis suppurativa); PAPASH (pyogenic arthritis, acne, PG and hidradenitis suppurativa); Sweet syndrome (SS); subcorneal pustular dermatosis (SPD); epidermolysis bullosa acquisita, erythema elevatum diutinum (EED); neutrophilic panniculitis; bowel-associated dermatosis-arthritis syndrome (BADAS); SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis)
25 syndrome; rheumatoid neutrophilic dermatosis; familial Mediterranean fever, cryopyrin-associated disorders, gout, and Schnitzler syndrome.

As used herein, the expression “HS-related disease” comprises without limitation Pyoderma gangrenosum (PG); PAPA (pyogenic arthritis, PG and acne); PASH (PG, acne and hidradenitis suppurativa); PAPASH (pyogenic arthritis, acne, PG and hidradenitis suppurativa);
30 Sweet syndrome (SS); and subcorneal pustular dermatosis (SPD).

IFX-1 (alternative name: CaCP29; InflaRx GmbH, Germany) is an antibody specifically binding to C5a. The CDR sequences and FR sequences of IFX-1 are disclosed in WO 2015/140304 A1 (Table 3), the content of which is hereby incorporated by reference in its entirety.

INab708 (InflaRx GmbH, Germany) is another antibody specifically binding to C5a. The CDR sequences and FR sequences of INab708 are also disclosed in WO 2015/140304 A1 (Table 3), the content of which is incorporated by reference in its entirety.

MEDI-7814 (MedImmune) is a recombinant humanized anti-C5a antibody. The crystal structure of the human C5a in complex with MEDI7814 is available in the RCSB Protein Data Bank under 4UU9 (DOI: 10.2210/pdb4uu9/pdb).

ALXN-1007 (Alexion) is a humanized anti-C5a antibody.

NOX-D21 (Noxxon) is a PEGylated mixed L-RNA/DNA-aptamer (Spiegelmer™) with the sequence 40 kDaPEG-aminoethyl-GCG AUG (dU)GG UGG UGA AGG GUU GUU GGG (dU)GU CGA CGC A(dC)G C (SEQ ID NO: 34). NOX-D21 targets C5a (Hyzewicz J, Tanihata J, Kuraoka M, Nitahara-Kasahara Y, Beylier T, Ruegg UT, Vater A, and Takeda S. 2017. *Low-Intensity Training and the C5a Complement Antagonist NOX-D21 Rescue the mdx Phenotype through Modulation of Inflammation*. Am. J. Pathol., 187(5):1147-1161; electronically published ahead of print: March 18, 2017).

Eculizumab (Alternative names: Soliris™, 5G1-1; h5G1.1; Alexion Pharmaceuticals) is a recombinant humanized monoclonal IgG2/4κ antibody produced by murine myeloma cell culture and purified by standard bioprocess technology. Eculizumab specifically binds to human C5. Eculizumab contains human constant regions from human IgG2 sequences and human IgG4 sequences and murine complementarity-determining regions grafted onto the human framework light- and heavy-chain variable regions. Eculizumab is composed of two 448 amino acid heavy chains and two 214 amino acid light chains and has a molecular weight of approximately 148 kDa. The heavy chain and light chain of eculizumab are disclosed, for example, in WO 2016/061066 A1 as SEQ ID NO: 1 and SEQ ID NO: 34, respectively. Nucleic acids that encode the heavy and light chains of eculizumab are disclosed, for example, in U.S. Patent No. 6,355,245.

ALXN1210 (Alternative name: BNJ441; Alexion Pharmaceuticals) is an anti-C5 antibody. The heavy and light chains of ALXN1210 are disclosed in WO 2016/209956 A1 as SEQ ID NOs: 14 and 11, respectively.

ALXN5500 (Alexion) is a humanized anti-C5 antibody. It is a next-generation eculizumab candidate.

LFG316 (Alternative name: Tesidolumab, NOV-4; Morphosys, Novartis) is an anti-C5 antibody.

Coversin™ (alternative names: EV 576; PAS-coversin; rEV 576; Tissue targeted Coversin™ - Akari; Akari Therapeutics, Evolutec) is a recombinant protein molecule

(16.7 kDa) derived from a salivary molecule from the *Ornithodoros moubata* tick where it assists the parasite to feed without provoking a host immunological response. The amino acid sequence of the EV576 protein (i.e. Coversin) as well as its coding nucleotide sequence are shown in Fig. 2 of WO 2008/029167. CoversinTM binds to C5.

5 RA101495 (Ra Pharma) is a macrocyclic synthetic peptide inhibitor of C5 (Ricardo A, Arata M, DeMarco S, Dhamnaskar K, Hammer R, Fridkis-Hareli M, Rajagopal V, Seyb K, Tang G-Q, Tobe S and Treco D. 2015. *Preclinical Evaluation of RA101495, a Potent Cyclic Peptide Inhibitor of C5 for the Treatment of Paroxysmal Nocturnal Hemoglobinuria*. Blood 126:939).

10 Zimura® (Alternative names: Anti-C5 aptamer; ARC-187; ARC-1905; Avacincaptad pegol sodium; OphthoTech Corporation, Archemix Corporation) is a pegylated RNA aptamer that inhibits complement factor C5. The nucleotide sequence of ARC1905 (i.e. Zimura) is shown, for example, in WO 2005/079363 A2 as SEQ ID NO: 67, and its structure is shown in Fig. 22 of WO 2005/079363 A2.

15 AMY-201 (Amyndas Pharmaceuticals) is an engineered form of Factor H that directly links the regulatory and surface-recognition domains; thus, it is a sort of mini-FH molecule.

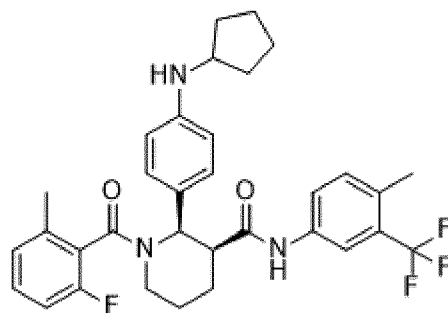
Mirococept (alternative names: APT070 and APT 070C; originator: Adprotech; developer: Inflazyme Pharmaceuticals) consists of the first three short consensus domains of human complement receptor 1, manufactured in recombinant bacteria and modified with a
20 membrane-targeting amphiphilic peptide based on the naturally occurring membrane-bound myristoyl-electrostatic switch peptide (Souza DG, Esser D, Bradford R, Vieira AT, and Teixeira MM. 2005. *APT070 (Mirococept), a membrane-localised complement inhibitor, inhibits inflammatory responses that follow intestinal ischaemia and reperfusion injury*. Br J Pharmacol 145(8):1027-1034).

25 BikacioMab (Novelmed) is an F(ab)₂ fragment of an anti-factor Bb antibody termed NM001. Antibody NM001 is produced by hybridoma cell line 1D3 deposited under ATCC accession number PTA-8543.

Lampalizumab (alternative names: Anti-factor D Fab; FCFD4514S; RG7417; TNX-234; originator: Tanox, Developer: Genentech) is a humanized anti-Factor D Fab fragment that
30 inhibits Factor D and the alternative complement pathway, through binding to an exosite on factor D.

ALN-CC5 (Alnylam) is an RNAi therapeutic targeting human, primate and rodent C5. Exemplary iRNA compositions targeting the C5 gene are described in WO 2016/044419.

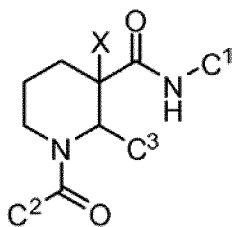
Avacopan (also known by the name CCX168; Chemocentryx) is a small molecule (MW = 581.66 g/mol) that has a structure according to formula I:



I

The IUPAC/Chemical name of avacopan is (2R,3S)-2-[4-(cyclopentylamino)phenyl]-1-(2-fluoro-6-methylbenzoyl)-N-[4-methyl-3-(trifluoromethyl)phenyl]piperidine-3-carboxamide. Avacopan is a selective inhibitor of C5aR. In the context of the present invention, the term “avacopan” refers to the compound according to formula I as well as to physiologically tolerable salts thereof.

Compounds similar to Avacopan that are also suitable for practicing the present invention are disclosed in international patent applications WO 2010/075257 A1 and WO 2011/163640 A1, the contents of which are herein incorporated by reference in their entirety. Thus, in some embodiments the inhibitor of C5a activity is a compound having the formula II



II

and pharmaceutically acceptable salts, hydrates and rotomers thereof; wherein

C¹ is selected from the group consisting of aryl and heteroaryl, wherein the heteroaryl group has from 1-3 heteroatoms as ring members selected from N, O and S; and wherein said aryl and heteroaryl groups are optionally substituted with from 1 to 3 R¹ substituents;

C² is selected from the group consisting of aryl and heteroaryl, wherein the heteroaryl group has from 1-3 heteroatoms as ring members selected from N, O and S; and wherein said aryl and heteroaryl groups are optionally substituted with from 1 to 3 R² substituents;

C³ is selected from the group consisting of C₁₋₈ alkyl or heteroalkyl, C₃₋₈ cycloalkyl, C₃₋₈ cycloalkyl-C₁₋₄ alkyl, aryl, aryl-C₁₋₄ alkyl, heteroaryl, heteroaryl-C₁₋₄ alkyl, heterocycloalkyl or heterocycloalkyl-C₁₋₄ alkyl, wherein the heterocycloalkyl group or portion has from 1-3 heteroatoms selected from N, O and S, and wherein the heteroaryl

group has from 1-3 heteroatoms as ring members selected from N, O and S, and each C³ is optionally substituted with from 1-3 R³ substituents;

each R¹ is independently selected from the group consisting of halogen, -CN, -R^c, -CO₂R^a, -CONR^aR^b, -C(O)R^a, -OC(O)NR^aR^b, -NR^bC(O)R^a, -NR^bC(O)R^a2R^c, -NR^a-C(O)NR^aR^b, -NR^aC(O)NR^aR^b, -NR^aR^b, -OR^a, and -S(O)₂NR^aR^b; wherein each R^a and R^b is independently selected from hydrogen, C₁₋₈ alkyl, and C₁₋₈ haloalkyl, or when attached to the same nitrogen atom can be combined with the nitrogen atom to form a five or six-membered ring having from 0 to 2 additional heteroatoms as ring members selected from N, O or S, and is optionally substituted with one or two oxo; each R^c is independently selected from the group consisting of C₁₋₈ alkyl or heteroalkyl, C₁₋₈ haloalkyl, C₃₋₆ cycloalkyl, heterocycloalkyl, aryl and heteroaryl, and wherein the aliphatic and cyclic portions of R^a, R^b and R^c are optionally further substituted with from one to three halogen, hydroxy, methyl, amino, alkylamino and dialkylamino groups; and optionally when two R¹ substituents are on adjacent atoms, are combined to form a fused five or six- membered carbocyclic or heterocyclic ring;

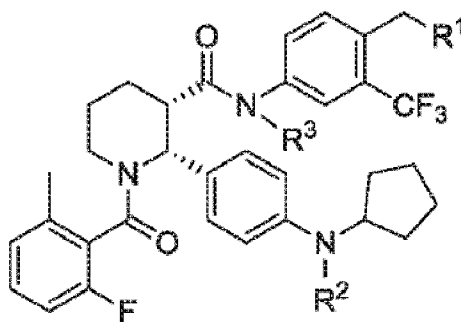
each R² is independently selected from the group consisting of halogen, -CN, -NO₂, -R^f, -CO₂R^d, -CONR^dR^e, -C(O)R^d, -OC(O)NR^dR^e, -NR^eC(O)R^d, -NR^eC(O)R^d2R^f, -NR^dC(O)NR^dR^e, -NR^dC(O)NR^dR^e, -NR^dR^e, -OR^d, and -S(O)₂NR^dR^e; wherein each R^d and R^e is independently selected from hydrogen, C₁₋₈ alkyl, and C₁₋₈ haloalkyl, or when attached to the same nitrogen atom can be combined with the nitrogen atom to form a five or six-membered ring having from 0 to 2 additional heteroatoms as ring members selected from N, O or S, and is optionally substituted with one or two oxo; each R is independently selected from the group consisting of C₁₋₈ alkyl or heteroalkyl, C₁₋₈ haloalkyl, C₃₋₆ cycloalkyl, heterocycloalkyl, aryl and heteroaryl, and wherein the aliphatic and cyclic portions of R^d, R^e and R^f are optionally further substituted with from one to three halogen, hydroxy, methyl, amino, alkylamino and dialkylamino groups, and optionally when two R² groups are on adjacent atoms, they are combined to form a five- or six-membered ring;

each R³ is independently selected from the group consisting of halogen, -CN, -Rⁱ, -CO₂R^g, -CONR^gR^h, -C(O)R^g, -C(O)Rⁱ, -OC(O)NR^gR^h, -NR^hC(O)R^g, -NR^hCO₂Rⁱ, -NR^gC(O)NR^gR^h, -NR^gR^h, -OR^g, -OR^j, -S(O)₂NR^gR^h, -X⁴-R^j, -NH-X⁴-R^j, -O-X⁴-R^j, -X⁴-NR^gR^h, -X⁴-NHR^j, -X⁴-CONR^gR^h, -X⁴-NR^hC(O)R^g, -X⁴-CO₂R^g, -O-X⁴-CO₂R^g, -NH-X⁴-CO₂R^g, -X⁴-NR^hCO₂Rⁱ, -O-X⁴-NR^hCO₂Rⁱ, -NHR^j and -NHCH₂R^j, wherein X⁴ is a C₁₋₄ alkylene; each R^g and R^h is independently selected from hydrogen, C₁₋₈ alkyl or

heteroalkyl, C₃₋₆ cycloalkyl and C₁₋₈ haloalkyl, or when attached to the same nitrogen atom can be combined with the nitrogen atom to form a four-, five- or six-membered ring having from 0 to 2 additional heteroatoms as ring members selected from N, O or S and is optionally substituted with one or two oxo; each Rⁱ is independently selected from the group consisting of C₁₋₈ alkyl or heteroalkyl, C₁₋₈ haloalkyl, C₃₋₆ cycloalkyl, heterocycloalkyl, aryl and heteroaryl; and each R^j is selected from the group consisting of C₃₋₆ cycloalkyl, imidazolyl, pyrimidinyl, pyrrolinyl, piperidinyl, morpholinyl, tetrahydrofuranyl, tetrahydropyranyl, and S,S-dioxo-tetrahydrothiopyranyl, and wherein the aliphatic and cyclic portions of R^g, R^h, Rⁱ and R^j are optionally further substituted with from one to three halogen, methyl, CF₃, hydroxy, C₁₋₄ alkoxy, C₁₋₄ alkoxy-C₁₋₄ alkyl, -C(O)O-C₁₋₈ alkyl, amino, alkylamino and dialkylamino groups, and optionally when two R³ groups are on adjacent atoms, they are combined to form a five- or six-membered ring; and

X is hydrogen or CH₃.

Compounds that are similar to Avacopan but have an improved solubility profile are disclosed in WO 2017/176620 A2, the content of which is herein incorporated by reference in its entirety. Thus, in some other embodiments the inhibitor of C5a activity is a compound of the following formula III:



III

or a pharmaceutically acceptable salt thereof, wherein:

R¹ is selected from the group consisting of H, -O-CH₂-O-P(O)OR^aOR^b, -O-C(O)-C₁₋₆ alkylene-L²-X¹, O-P(O)OR^aOR^b, and -O-C(O)-A¹-(C₁₋₃ alkylene)_n-C₄₋₇ heterocyclyl wherein the C₄₋₇ heterocyclyl is optionally substituted with 1 to 6 R^c groups;

A¹ is selected from the group consisting of C₆₋₁₀ aryl, C₃₋₁₀ cycloalkyl, C₅₋₁₀ heteroaryl and C₅₋₁₀ heterocyclyl, each of which is optionally substituted with 1 to 5 R^x which can be the same or different;

n=0 or 1;

L^2 is independently selected from the group consisting of a bond, $-O-C(O)-C_{1-6}$ alkylene-, and $-NR^d-C(O)-C_{1-6}$ alkylene-;

X^1 is independently selected from the group consisting of $-NR^eR^f$, $-P(O)OR^aOR^b$, $-O-P(O)OR^aOR^b$, and $-CO_2H$;

5 R^2 is selected from the group consisting of H, $-L^3-C_{1-6}$ alkylene- L^4-X^2 , $-L^3-(C_{1-6} \text{ alkylene})_m-A^2-X^2$, $-P(O)OR^aOC(O)-C_{1-6}$ alkyl, $-P(O)OR^aNR^gR^h$ and $-P(O)OR^aOR^b$;

L^3 is independently selected from the group consisting of $-C(O)-O-$, and $-C(O)-$;

10 L^4 is independently selected from the group consisting of a bond, $-O-C(O)-C_{2-6}$ alkenylene-, $-O-C(O)-C_{1-6}$ alkylene-, and $-NR^d-C(O)-C_{1-6}$ alkylene- wherein the C_{1-6} alkylene in $-NR^d-C(O)-C_{1-6}$ alkylene- and $-O-C(O)-C_{1-6}$ alkylene- is optionally substituted with NR^eR^f ;

X^2 is independently selected from the group consisting of $-NR^kR^l$, $-P(O)OR^aOR^b$, $-O-P(O)OR^aOR^b$, and $-CO_2H$;

$m=0$ or 1 ;

15 A^2 is selected from the group consisting of C_{6-10} aryl, C_{3-10} cycloalkyl, C_{5-10} heteroaryl and C_{5-10} heterocyclyl, each of which is optionally substituted with 1 to 5 R^x which can be the same or different;

R^3 is H or $-L^5-P(O)OR^aOR^b$ wherein L^5 is independently selected from the group consisting of a bond and $-CH_2-O-$;

20 each R^x is independently selected from the group consisting of halogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} heteroalkyl, CN, NR^yR^z , SR^y and OR^y ;

each R^c is independently selected from the group consisting of halogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} heteroalkyl, CN, NR^yR^z , SR^y and OR^y ;

25 each R^a , R^b , R^d , R^e , R^f , R^g , R^k , R^l , R^y and R^z is independently selected from the group consisting of H and C_{1-6} alkyl;

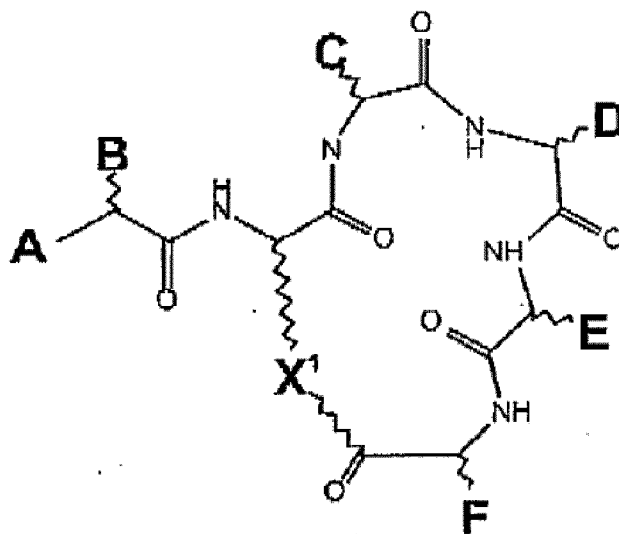
each R^h is independently selected from the group consisting of H and C_{1-6} alkyl wherein the C_{1-6} alkyl is optionally substituted with 1 to 5 substituents independently selected from CO_2H , NR^iR^j , C_{6-10} aryl, C_{3-10} cycloalkyl, C_{5-10} heteroaryl and C_{5-10} heterocyclyl, wherein each R^i and R^j is independently H or C_{1-6} alkyl;

30 wherein two of R^1 , R^2 and R^3 are H, and one of R^1 , R^2 and R^3 is other than H.

PMX-53 is a potent antagonist of C5aR (CD88). It is a circular peptide composed of six amino acids, with the following sequence: Ac-Phe-cyclo(Orn-Pro-D-Cha-Trp-Arg) with a lactam bridge between Orn-2 and Arg-6. Since PMX-53 contains at least one D-amino acid (i.e. D-Cha), it is not included the enclosed sequence listing of this application. PMX-53 is

commercially available by bio-technie GmbH (Wiesbaden-Nordenstadt, Germany), Cat. No. 5473.

Compounds similar to PMX-53 that are also suitable for practicing the present invention are disclosed in international patent applications WO 99/00406 A1, WO 03/033528 A1, and
 5 WO 2008/009062 A1, which are herein incorporated by reference in their entirety. Thus, in some embodiments the inhibitor of C5a activity is a cyclic peptide or peptidomimetic compound of the formula IV



IV

- 10 where A is H, alkyl, aryl, NH₂, NH-alkyl, N(alkyl)₂, NH-aryl, NH-acyl, NH-benzoyl, NHSO₃, NHSO₂-alkyl, NHSO₂-aryl, OH, O-alkyl, or O-aryl;
 B is an alkyl, aryl, phenyl, benzyl, naphthyl or indole group, or the side chain of a D- or L-amino acid, but is not the side chain of glycine, D-phenylalanine, L-homophenylalanine, L-tryptophan, L-homotryptophan, L-tyrosine, or L-homotyrosine;
 15 C is the side chain of a D-, L- or homo-amino acid, but is not the side chain of isoleucine, phenylalanine, or cyclohexylalanine;
 D is the side chain of a neutral D-amino acid, but is not the side chain of glycine or D-alanine, a bulky planar side chain, or a bulky charged side chain;
 E is a bulky substituent, but is not the side chain of D-tryptophan, L-N-methyltryptophan, L-
 20 homophenylalanine, L-2-naphthyl L-tetrahydroisoquinoline, L-cyclohexylalanine, D-leucine, L-fluorenylalanine, or L-histidine;
 F is the side chain of L-arginine, L-homoarginine, L-citrulline, or L-canavanine, or a bioisostere thereof; and

X¹ is -(CH₂)_nNH- or (CH₂)_nS-, where n is an integer of from 1 to 4; -(CH₂)₂O-; -(CH₂)₃O-; -(CH₂)₃-; -(CH₂)₄-; -CH₂-COCHRNH-; or -CH₂-CHCOCHRNH-, where R is the side chain of any common or uncommon amino acid.

In this context, the term “common amino acid” refers to the twenty proteinogenic amino acids that are defined by the standard genetic code. The term “uncommon amino acid” includes, but is not restricted to, 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, ornithine, citrulline, canavanine, norleucine, δ-glutamic acid, aminobutyric acid, L-fluorenylalanine, L-3-benzothienylalanine, and α,α-disubstituted amino acids.

Specific antagonists of C5aR (CD88) suitable for practicing the present invention include PMX95, PMX218, PMX200, PMX273, PMX205, and PMX201, as disclosed in WO 2008/009062 A1.

Clone S5/1 is a monoclonal antibody recognizing the human receptor for C5a (CD88). Clone S5/1 was raised against a synthetic peptide comprising the N-terminal domain of the C5aR (Met1-Asn31). The antibody has been shown to inhibit the binding of C5a to its receptor. It is commercially available via Hycult Biotech (Uden, The Netherlands), Cat. No. HM2094.

Clone 7H110 is a monoclonal mouse antibody recognizing the human receptor for C5a (CD88). It is commercially available via Biomol GmbH (Hamburg, Germany); Cat. No. C2439-60N.

As used herein, a “patient” means any mammal or bird who may benefit from a treatment with the compound described herein (i.e. with an inhibitor of C5a activity described herein). Preferably, a “patient” is selected from the group consisting of laboratory animals (e.g. mouse or rat), domestic animals (including e.g. guinea pig, rabbit, chicken, turkey, pig, sheep, goat, camel, cow, horse, donkey, cat, or dog), or primates including chimpanzees and human beings. It is particularly preferred that the “patient” is a human being.

As used herein, “treat”, “treating” or “treatment” of a disease or disorder means accomplishing one or more of the following: (a) reducing the severity and/or duration of the disorder; (b) limiting or preventing development of symptoms characteristic of the disorder(s) being treated; (c) inhibiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting or preventing recurrence of the disorder(s) in patients that have previously had the disorder(s); and (e) limiting or preventing recurrence of symptoms in patients that were previously symptomatic for the disorder(s).

As used herein, “prevent”, “preventing”, “prevention”, or “prophylaxis” of a disease or disorder means preventing that a disorder occurs in a subject.

An “effective amount” is an amount of a therapeutic agent sufficient to achieve the intended purpose. The effective amount of a given therapeutic agent will vary with factors such as the nature of the agent, the route of administration, the size and species of the animal to receive the therapeutic agent, and the purpose of the administration. The effective amount in each individual case may be determined empirically by a skilled artisan according to established methods in the art.

“Pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

Embodiments of the Invention

The present invention will now be further described. In the following passages different aspects of the invention are defined in more detail. Each aspect defined below may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

In a first aspect the present invention is directed to a compound for use in the treatment of a cutaneous, neutrophilic, inflammatory disease in a subject, wherein the compound is an inhibitor of C5a activity, and wherein the cutaneous, neutrophilic, inflammatory disease is selected from the group consisting of hidradenitis suppurativa (HS); Pyoderma gangrenosum (PG); PAPA (pyogenic arthritis, PG and acne); PASH (PG, acne and hidradenitis suppurativa); PAPASH (pyogenic arthritis, acne, PG and hidradenitis suppurativa); Sweet syndrome (SS); subcorneal pustular dermatosis (SPD); epidermolysis bullosa acquisita, erythema elevatum diutinum (EED); neutrophilic panniculitis; bowel-associated dermatosis-arthritis syndrome (BADAS); SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis) syndrome; rheumatoid neutrophilic dermatosis; familial Mediterranean fever, cryopyrin-associated disorders, gout, and Schnitzler syndrome.

In a second aspect, the present invention is directed to a method for the treatment of a cutaneous, neutrophilic, inflammatory disease in a subject, comprising the step of:

administering to a subject in need thereof a therapeutic amount of a compound, wherein the compound is an inhibitor of C5a activity, and wherein the cutaneous, neutrophilic, inflammatory disease is selected from the group consisting of hidradenitis suppurativa (HS);

Pyoderma gangrenosum (PG); PAPA (pyogenic arthritis, PG and acne); PASH (PG, acne and hidradenitis suppurativa); PAPASH (pyogenic arthritis, acne, PG and hidradenitis suppurativa); Sweet syndrome (SS); subcorneal pustular dermatosis (SPD); epidermolysis bullosa acquisita, erythema elevatum diutinum (EED); neutrophilic panniculitis; bowel-associated dermatosis-arthritis syndrome (BADAS); SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis) syndrome; rheumatoid neutrophilic dermatosis; familial Mediterranean fever, cryopyrin-associated disorders, gout, and Schnitzler syndrome.

In a third aspect, the present invention is directed to a use of a compound for the preparation of a pharmaceutical composition for the treatment of a cutaneous, neutrophilic, inflammatory disease, wherein the compound is an inhibitor of C5a activity, and wherein the cutaneous, neutrophilic, inflammatory disease is selected from the group consisting of hidradenitis suppurativa (HS); Pyoderma gangrenosum (PG); PAPA (pyogenic arthritis, PG and acne); PASH (PG, acne and hidradenitis suppurativa); PAPASH (pyogenic arthritis, acne, PG and hidradenitis suppurativa); Sweet syndrome (SS); subcorneal pustular dermatosis (SPD); epidermolysis bullosa acquisita, erythema elevatum diutinum (EED); neutrophilic panniculitis; bowel-associated dermatosis-arthritis syndrome (BADAS); SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis) syndrome; rheumatoid neutrophilic dermatosis; familial Mediterranean fever, cryopyrin-associated disorders, gout, and Schnitzler syndrome.

In some embodiments of any aspect of the present invention, the inhibitor of C5a activity:

- lowers the concentration of C5 (for example, by inhibiting formation and/or activity of C3 convertase; by inhibiting formation and/or activity of C5 convertase; by inhibiting the transcription of the C5 gene; by blocking translation of the C5 mRNA; by increasing degradation of the C5 mRNA; by increasing degradation of the C5 protein; or by prevention secretion of C5 from the liver);
- inhibits the cleavage of C5 into C5a and C5b (for example, by inhibiting the C5 convertase or by binding to a cleavage site on C5 thereby blocking cleavage);
- lowers the concentration of C5a (for example, by increasing degradation of the C5a protein);
- inhibits the binding between C5a and a C5a receptor (for example by binding to C5a or by binding to a C5a receptor);
- lowers the concentration of a C5a receptor (for example, by inhibiting transcription of a C5a receptor gene; by blocking translation of a C5a receptor mRNA; by increasing degradation of a C5a receptor mRNA; by increasing degradation of a C5a receptor protein); and/or

- inhibits the activity of a C5a receptor.

In some embodiments of any aspect of the present invention, the inhibitor of C5a activity is selected from the group consisting of a protein ligand (as defined above); an oligonucleotide; and a small molecule (as defined above). Oligonucleotides acting as inhibitors of C5a activity can achieve their inhibitory effect for example by binding to nucleic acid molecules (thereby inhibiting transcription and/or translation) or by binding to proteins (e.g. when the oligonucleotides are nucleic acid aptamers).

In some embodiments of any aspect of the present invention, the inhibitor of C5a activity is a protein ligand that specifically binds to C5 protein, or to C5a protein, or to a C5a receptor protein. In further embodiments, the protein ligand is selected from the group consisting of

- (i) antibodies (e.g. anti-C5 antibodies, anti-C5a antibodies, anti-C5aR antibodies, or anti-C5L2 antibodies),
- (ii) antigen-binding fragments of antibodies,
- (iii) antibody-like proteins,
- (iv) inhibitory variants of C5a,
- (v) inhibitory variants of a C5a receptor (e.g. decoy receptors),
- (vi) proteins acting on the complement pathway (e.g. Coversin); and
- (vii) peptides (e.g. RA101495 (Ra Pharma, Cambridge, MA); PMX-53 (bio-technie GmbH (Wiesbaden-Nordenstadt, Germany))).

In some embodiments of any aspect of the present invention, the inhibitor of C5a activity is a protein ligand or an oligonucleotide, preferably a protein ligand, that specifically binds to a conformational epitope formed by amino acid sequences NDETCEQRA (SEQ ID NO: 2) and SHKDMQL (SEQ ID NO: 3) of human C5a. Binding to the conformational formed by the amino acid sequences according to SEQ ID NOs: 2 and 3 means that the protein ligand or oligonucleotide binds to at least one amino acid within the amino acid sequence according to SEQ ID NO: 2 and to at least one amino acid within the amino acid sequence according to SEQ ID NO: 3. SEQ ID NO: 2 corresponds to amino acids 30-38 of human C5a. SEQ ID NO: 3 corresponds to amino acids 66-72 of human C5a.

In some embodiments of any aspect of the present invention the protein ligand or oligonucleotide, preferably the protein ligand, binds to at least one amino acid within the amino acid sequence according to DETCEQR (SEQ ID NO: 4). SEQ ID NO: 4 corresponds to amino acids 31-37 of human C5a.

In some embodiments of any aspect of the present invention the protein ligand or oligonucleotide, preferably the protein ligand, binds to at least one amino acid within the amino

acid sequence according to HKDMQ (SEQ ID NO: 5), more preferably to at least one amino acid within the amino acid sequence KDM. SEQ ID NO: 5 corresponds to amino acids 67-71 of human C5a; the sequence KDM corresponds to amino acids 68-70 of human C5a.

5 In some embodiments of any aspect of the present invention the protein ligand or oligonucleotide, preferably the protein ligand, binds to at least one amino acid within the amino acid sequence DETCEQR (SEQ ID NO: 4) and to at least one amino acid within the amino acid sequence HKDMQ (SEQ ID NO: 5).

10 In some embodiments of any aspect of the present invention the protein ligand or oligonucleotide, preferably the protein ligand, binds to at least one amino acid within the amino acid sequence DETCEQR (SEQ ID NO: 4) and to at least one amino acid within the amino acid sequence KDM.

15 In some embodiments of any aspect of the present invention the two sequences forming the conformational epitope of C5a (e.g. sequence pairs according to SEQ ID NO: 2 and 3; SEQ ID NO: 4 and 5; or SEQ ID NO: 4 and sequence KDM) are separated by 1-50 contiguous amino acids that do not participate in binding to the binding moiety of the invention. In the following, such amino acids that do not participate in binding to the binding moiety of the invention will be referred to as “non-binding amino acids”. The two sequences forming the conformational epitope are preferably separated by 6-45 contiguous non-binding amino acids, more preferably by 12-40 contiguous non-binding amino acids, more preferably by 18-35 contiguous non-binding amino acids, more preferably by 24-30 contiguous non-binding amino acids, more preferably by 25-29 contiguous non-binding amino acids, even more preferably by 26-28 contiguous non-binding amino acids, and most preferably by 27 contiguous non-binding amino acids.

25 In some embodiments of any aspect of the present invention the protein ligand or oligonucleotide, preferably the protein ligand, specifically binding to a conformational epitope of C5a has a binding constant to human C5a with a K_d value of 10 nM or less, preferably 9 nM or less, more preferably 8 nM or less, more preferably 7 nM or less, more preferably 6 nM or less, more preferably 5 nM or less, more preferably 4 nM or less, more preferably 3 nM or less, more preferably 2 nM or less, and even more preferably 1 nM or less. In some embodiments of
30 any aspect of the present invention the dissociation constant K_d between the binding moiety and human C5a is between 1 pM (picomolar) and 5 nM (nanomolar), more preferably between 2 pM and 4 nM, more preferably between 5 pM and 3 nM, more preferably between 10 pM and 2 nM, more preferably between 50 pM and 1 nM, more preferably between 100 pM and 900

pM, more preferably between 200 pM and 800 pM, more preferably between 300 pM and 700 pM, and even more preferably between 400 pM and 600 pM.

In some embodiments of any aspect of the present invention the protein ligand or oligonucleotide, preferably the protein ligand, specifically binding to C5a exhibits at least 75% blocking activity, preferably at least 80% blocking activity, more preferably at least 85% blocking activity, more preferably at least 90% blocking activity, more preferably at least 95% blocking activity for biological effects induced by one molecule C5a, particularly human C5a. These particular blocking activities refer to those embodiments, wherein the binding moiety comprises a single paratope binding to C5a, preferably human C5a. In embodiments, wherein the binding moiety comprises two or more C5a-specific paratopes, said blocking activities of at least 75%, preferably at least 80%, more preferably at least 85%, etc. are achieved when one binding-moiety molecule is contacted with a number of C5a molecules equal to the number of C5a-specific paratopes present in the binding moiety. In other words, when the paratopes of a binding moiety described herein and C5a are present in equimolar concentrations, the binding moiety exhibits at least 75% blocking activity, preferably at least 80% blocking activity, more preferably at least 85% blocking activity, more preferably at least 90% blocking activity, and more preferably at least 95% blocking activity for biological effects induced by C5a. A preferred biological effect to be blocked is C5a-induced lysozyme release from human whole blood cells. Assays for determining this C5a-induced lysozyme release and its blocking are described, for example, in WO 2011/063980 A1 and in the corresponding US national stage application US 2012/0231008 A1.

In some embodiments of any aspect of the present invention the protein ligand is an antibody or an antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof comprises

- (i) a heavy chain CDR3 sequence as set forth in SEQ ID NO: 6; or
 - (ii) a heavy chain CDR3 sequence as set forth in SEQ ID NO: 7;
- wherein the heavy chain CDR3 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions.

In some embodiments of any aspect of the present invention the protein ligand is an antibody or an antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof comprises

- (iii) a light chain CDR3 sequence as set forth in SEQ ID NO: 8; or
- (iv) a light chain CDR3 sequence as set forth in SEQ ID NO: 9;

wherein the light chain CDR3 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions.

In some embodiments of any aspect of the present invention the protein ligand is an antibody or an antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof comprises

(i) a heavy chain CDR3 sequence as set forth in SEQ ID NO: 6 and a light chain CDR3 sequence as set forth in SEQ ID NO: 8; or

(ii) a heavy chain CDR3 sequence as set forth in SEQ ID NO: 7 and a light chain CDR3 sequence as set forth in SEQ ID NO: 9;

wherein the heavy chain CDR3 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions; and

wherein the light chain CDR3 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions.

In some embodiments of any aspect of the present invention the protein ligand is an antibody or an antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof comprises at least one of the following sequences:

(v) a heavy chain CDR2 sequence according to SEQ ID NO: 10;

(vi) a heavy chain CDR2 sequence according to SEQ ID NO: 11;

(vii) a light chain CDR2 sequence according to SEQ ID NO: 12;

(viii) a light chain CDR2 sequence according to SEQ ID NO: 13;

(ix) a heavy chain CDR1 sequence according to SEQ ID NO: 14;

(x) a heavy chain CDR1 sequence according to SEQ ID NO: 15;

(xi) a light chain CDR1 sequence according to SEQ ID NO: 16; or

(xii) a light chain CDR1 sequence according to SEQ ID NO: 17;

wherein the heavy chain CDR2 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions;

wherein the light chain CDR2 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions;

wherein the heavy chain CDR1 sequence optionally comprises 1, 2 or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions; and

wherein the light chain CDR1 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions.

In particular embodiments, the total number of these optional changes recited above in each one of the amino acid sequences according to SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17, i.e. the total number of exchanges, deletions and additions in each sequence, is 1 or 2.

In particular embodiments the total number of exchanges, deletions, and additions added up for all CDRs present in an antibody or antigen-binding fragment thereof is between 1 and 5 (e.g. 1, 2, 3, 4, or 5).

In some embodiments of any aspect of the present invention the protein ligand is an antibody or an antigen-binding fragment thereof, comprises one of the sets A to H of heavy chain CDR3, heavy chain CDR2, and heavy chain CDR1 sequences as listed below in Table 1, wherein each heavy chain CDR3 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions;

wherein each heavy chain CDR2 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions; and

wherein each heavy chain CDR1 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions:

Table 1: Sets of heavy chain CDR sequences suitable for use in the antibodies or fragments thereof of the present invention

Symbol of heavy chain set	CDR3 sequence	CDR2 sequence	CDR1 sequence
A	SEQ ID NO: 6	SEQ ID NO: 10	SEQ ID NO: 14
B	SEQ ID NO: 6	SEQ ID NO: 10	SEQ ID NO: 15

C	SEQ ID NO: 6	SEQ ID NO: 11	SEQ ID NO: 14
D	SEQ ID NO: 6	SEQ ID NO: 11	SEQ ID NO: 15
E	SEQ ID NO: 7	SEQ ID NO: 10	SEQ ID NO: 14
F	SEQ ID NO: 7	SEQ ID NO: 10	SEQ ID NO: 15
G	SEQ ID NO: 7	SEQ ID NO: 11	SEQ ID NO: 14
H	SEQ ID NO: 7	SEQ ID NO: 11	SEQ ID NO: 15

In some embodiments of any aspect of the present invention the protein ligand is an antibody or an antigen-binding fragment thereof, comprises one of the following sets I to IV of light chain CDR3, light chain CDR2, and light chain CDR1 sequences as listed in Table 2,

5 wherein each light chain CDR3 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions;

wherein each light chain CDR2 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions; and

10 wherein each light chain CDR1 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions.

15 **Table 2: Sets of light chain CDR sequences suitable for use in the antibodies or fragments thereof of the present invention**

Since the CDR2 light chain sequence of antibody IFX-1 (SEQ ID NO: 12) is identical to the CDR2 light chain sequence of antibody INab708 (SEQ ID NO: 13), sets including SEQ ID NO: 13 would be redundant to sets including SEQ ID NO: 12. Therefore, the table only lists four sets of light chain CDR sequences.

Number of light chain set	CDR3 sequence	CDR2 sequence	CDR1 sequence
I	SEQ ID NO: 8	SEQ ID NO: 12	SEQ ID NO: 16
II	SEQ ID NO: 8	SEQ ID NO: 12	SEQ ID NO: 17
III	SEQ ID NO: 9	SEQ ID NO: 12	SEQ ID NO: 16
IV	SEQ ID NO: 9	SEQ ID NO: 12	SEQ ID NO: 17

In some embodiments of any aspect of the present invention the protein ligand is an antibody or an antigen-binding fragment thereof, comprises one of the heavy CDR sets A-H listed above in Table 1 and one of the light chain CDR sets I-IV listed above in Table 2, i.e. one of the following combinations of sets: A-I, A-II, A-III, A-IV, B-I, B-II, B-III, B-IV, C-I, C-II, C-III, C-IV, D-I, D-II, D-III, D-IV, E-I, E-II, E-III, E-IV, F-I, F-II, F-III, F-IV, G-I, G-II, G-III, G-IV, H-I, H-II, H-III, or H-IV (wherein the combinations A-I and H-IV are especially preferred),

wherein each heavy chain CDR3 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions;

wherein each heavy chain CDR2 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions;

wherein each heavy chain CDR1 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions and/or 1, 2, or 3 amino acid additions;

wherein each light chain CDR3 sequence optionally comprises 1, 2, or 3 amino acid exchanges, in particular conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions;

wherein each light chain CDR2 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions, and/or 1, 2, or 3 amino acid additions; and

wherein each light chain CDR1 sequence optionally comprises 1, 2, or 3 amino acid exchanges, preferably conservative amino acid exchanges, 1, 2, or 3 amino acid deletions and/or 1, 2, or 3 amino acid additions.

In some embodiments of any aspect of the present invention the protein ligand is an antibody or an antigen-binding fragment thereof, comprises a VH domain that comprises, essentially consists of or consists of (i) the VH domain of IFX-1 or (ii) the VH domain of INab708.

The FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4 sequences defining the VH domains of IFX-1 and INab708 are shown below in Table 3.

In some embodiments of any aspect of the present invention the protein ligand is an antibody or an antigen-binding fragment thereof, comprises a VL domain that comprises,

essentially consists of or consists of (i) the VL domain of IFX-1 or (ii) the VL domain of INab708.

The FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4 sequences defining the VL domains of IFX-1 and INab708 are shown below in Table 3.

5

Table 3: CDR and FR sequences of antibodies IFX-1 and INab708 (Chothia classification mode)

IFX-1:	INab708:
<u>Heavy Chain:</u>	<u>Heavy Chain:</u>
FR1: QVQLQQSGPQLVRPGTSVKIS (= SEQ ID NO: 18)	FR1: VQLLESGAELMKPGASVKIS (SEQ ID NO: 26)
CDR1: CKASGYSFTTFWMD (= SEQ ID NO: 14)	CDR1: CKATGNTFSGYWIE (= SEQ ID NO: 15)
FR2: WVKQRPGQGLEWIGR (SEQ ID NO: 19)	FR2: WVKQRPGHGLEWIGE (SEQ ID NO: 27)
CDR2: IDPSDSESRLDQ (= SEQ ID NO: 10)	CDR2: ILPGSGSTNYNE (= SEQ ID NO: 11)
FR3: RFKDRATLTVDKSSSTVYMQLSSTSE DSAVYY (SEQ ID NO: 20)	FR3: KFKGKATLTADTSSNTAYMQLSSTSE DSAVYY (SEQ ID NO: 28)
CDR3: CARGNDGYYGFAY (= SEQ ID NO: 6)	CDR3: CTRRGLYDGSSYFAY (= SEQ ID NO: 7)
FR4: WGQGTLLTVSS (SEQ ID NO: 21)	FR4: WGQGTLLTVSA (SEQ ID NO: 29)

Light Chain:FR1: DIVLTQSPASLA VSLGQRATIS
(SEQ ID NO: 22)CDR1: CKASQSV D YDGDSYMK
(= SEQ ID NO: 16)FR2: WYQQKPGQP PKLL
(SEQ ID NO: 23)CDR2: IYAASNL
(= SEQ ID NO: 12)FR3:
QSGIPARFSGSGSGTDFTLNIHPVEEEDA
ATYY
(SEQ ID NO: 24)CDR3: CQQSNEDPYT
(= SEQ ID NO: 8)FR4: FGGGTKLEIK
(SEQ ID NO: 25)Light Chain:FR1: DIVLTQSPASLA VSLGQRATIS
(SEQ ID NO: 30)CDR1: CKASQSV D YDGDSYMN
(= SEQ ID NO: 17)FR2: WYQQKPGQP PKLL
(SEQ ID NO: 31)CDR2: IYAASNL
(= SEQ ID NO: 13)FR3:
GSGIPARFSGSGSGTDFTLNIHPVEEE
VAATYY
(SEQ ID NO: 32)CDR3: CQQNNEDPLT
(= SEQ ID NO: 9)FR4: FGAGTLLELK
(SEQ ID NO: 33)

In some embodiments of any aspect of the present invention, the inhibitor of C5a activity is an oligonucleotide that specifically binds to C5, or to C5a, or to a C5a receptor. In further embodiments, the oligonucleotide is a nucleic acid aptamer. The nucleic acid aptamer may be selected from the group consisting of DNA-aptamers, D-RNA aptamers, and L-RNA aptamers (e.g., Spiegelmers™).

In some embodiments of any aspect of the present invention, the inhibitor of C5a activity reduces expression of C5 protein or a C5a receptor protein. In further embodiments, said inhibitor of C5a activity that reduces expression of C5 protein or a C5a receptor protein is an oligonucleotide selected from the group consisting of antisense DNA, antisense RNA, siRNA, and miRNA.

In some embodiments of any aspect of the present invention, the C5a receptor is C5aR and/or C5L2. In preferred embodiments of any aspect of the present invention, the C5a receptor is C5aR (also known as CD88 or C5aR1).

In some embodiments of any aspect of the present invention, the inhibitor of C5a activity is selected from the group consisting of:

- (a) IFX-1, INab708, MEDI-7814, ALXN-1007, or NOX-D21, or an antigen-binding fragment thereof;

- (b) an antibody or an antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof competes with one of the antibodies indicated under (a) for binding to C5a;
- (c) Eculizumab, ALXN1210, ALXN5500, or LFG316, or an antigen-binding fragment thereof;
- 5 (d) an antibody or an antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof competes with one of the antibodies indicated under (c) for binding to C5;
- (e) Coversin or RA101495;
- (f) an antibody or an antigen-binding fragment thereof or protein or macrocyclic peptide
10 wherein said antibody or antigen-binding fragment thereof or macrocyclic peptide competes with one of the or protein or peptides indicated under (e) for binding to C5;
- (g) Zimura;
- (h) an antibody or an antigen-binding fragment thereof or an aptamer, wherein said antibody or antigen-binding fragment thereof or aptamer competes with Zimura for binding to C5;
- 15 (i) AMY-201 or Mirococept;
- (j) an antibody or an antigen-binding fragment thereof or a protein wherein said antibody or antigen-binding fragment thereof or protein competes with one of the proteins indicated under (i) for binding to C3b;
- (k) Bikaciomab;
- 20 (l) an antibody or an antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof competes with Bikaciomab for binding to Factor B;
- (m) Lampalizumab;
- (n) an antibody or an antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof competes with Lampalizumab for binding to Factor D;
- 25 (o) ALN-CC5;
- (p) Avacopan or a compound according to formula II or III or PMX-53 or a compound according to formula IV;
- (q) an antibody or an antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof competes with avacopan or PMX-53 for binding to C5aR;
- 30 (r) clone S5/1 or clone 7H110, or an antigen-binding fragment thereof; and
- (s) an antibody or an antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof competes with one of the antibodies indicated under (r) for binding to C5aR.

In some embodiments of any aspect of the present invention, the cutaneous, neutrophilic, inflammatory disease is

- an auto-inflammatory disease (more precisely: a cutaneous, neutrophilic, auto-inflammatory disease); or
- 5 - an autoimmune disease with cutaneous inflammation (more precisely: an autoimmune disease with cutaneous, neutrophilic inflammation).

In some embodiments of any aspect of the present invention, the cutaneous, neutrophilic, inflammatory disease is an auto-inflammatory disease selected from the group consisting of hidradenitis suppurativa (HS); Pyoderma gangrenosum (PG); PAPA (pyogenic
10 arthritis, PG and acne); PASH (PG, acne and hidradenitis suppurativa); PAPASH (pyogenic arthritis, acne, PG and hidradenitis suppurativa); Sweet syndrome (SS); subcorneal pustular dermatosis (SPD); epidermolysis bullosa acquisita, erythema elevatum diutinum (EED); neutrophilic panniculitis; bowel-associated dermatosis-arthritis syndrome (BADAS); and SAPHO (synovitis, acne, pustulosis, hyperostosis, and osteitis) syndrome.

15 In some embodiments of any aspect of the present invention, the cutaneous, neutrophilic, inflammatory disease is HS or a HS-related disease selected from the group consisting of Pyoderma gangrenosum (PG); PAPA (pyogenic arthritis, PG and acne); PASH (PG, acne and hidradenitis suppurativa); PAPASH (pyogenic arthritis, acne, PG and hidradenitis suppurativa); Sweet syndrome (SS); and subcorneal pustular dermatosis (SPD).

20 In some embodiments of any aspect of the present invention, the cutaneous, neutrophilic, inflammatory disease is an autoimmune disease with cutaneous inflammation selected from the group consisting of rheumatoid neutrophilic dermatosis; familial Mediterranean fever, cryopyrin-associated disorders, gout, and Schnitzler syndrome.

In some embodiments of the first or third aspect of the present invention, the compound
25 is to be administered at a dose of 800 mg once per week or at a dose of 800 mg twice per week. In further embodiments of the first or third aspect,

- the inhibitor of C5a activity is a compound specifically binding to C5a (preferably selected from the group consisting of IFX-1, INab708, MEDI-7814, ALXN-1007, NOX-D21, and an antigen-binding fragment thereof; more preferably the inhibitor of C5a activity is
30 selected from the group consisting of IFX-1, INab708, MEDI-7814, ALXN-1007 and an antigen-binding fragment thereof; even more preferably, the inhibitor of C5a activity is selected from the group consisting of IFX-1 and an antigen-binding fragment thereof; most preferably the inhibitor of C5a activity is IFX-1); and
- the cutaneous, neutrophilic, inflammatory disease is hidradenitis suppurativa (HS); and

- the compound is to be administered at a dose of 800 mg once per week or at a dose of 800 mg twice per week.

In further embodiments of the first or third aspect, the inhibitor of C5a activity is to be administered intravenously. In further embodiments of the first or third aspect, the inhibitor of C5a activity is to be administered twice per week at a dose of 800 mg in the first week of treatment and once per week at a dose of 800 mg in the second and subsequent weeks of treatment. In further embodiments of the first or third aspect, the total duration of treatment is between 5 and 12 weeks (e.g. 5 weeks, 6, weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks).

In some embodiments of the second aspect of the present invention, the compound is administered at a dose of 800 mg once per week or at a dose of 800 mg twice per week. In further embodiments of the second aspect,

- the inhibitor of C5a activity is a compound specifically binding to C5a (preferably selected from the group consisting of IFX-1, INab708, MEDI-7814, ALXN-1007, NOX-D21, and an antigen-binding fragment thereof; more preferably the inhibitor of C5a activity is selected from the group consisting of IFX-1, INab708, MEDI-7814, ALXN-1007 and an antigen-binding fragment thereof; even more preferably, the inhibitor of C5a activity is selected from the group consisting of IFX-1 and an antigen-binding fragment thereof; most preferably the inhibitor of C5a activity is IFX-1); and
- the cutaneous, neutrophilic, inflammatory disease is hidradenitis suppurativa (HS); and
- the compound is administered at a dose of 800 mg once per week or at a dose of 800 mg twice per week.

In further embodiments of the second aspect, the inhibitor of C5a activity is administered intravenously. In further embodiments of the second aspect, the compound is administered twice per week at a dose of 800 mg in the first week of treatment and once per week at a dose of 800 mg in the second and subsequent weeks of treatment. In further embodiments of the second aspect, the total duration of treatment is between 5 and 12 weeks (e.g. 5 weeks, 6, weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, or 12 weeks).

Pharmaceutical compositions and Modes of Administration

In the practice of any aspect of the present invention, a compound (e.g. an inhibitor of C5a activity described herein) or a pharmaceutical composition comprising the compound may be administered to a patient by any route established in the art which provides a sufficient level of the compound in the patient. It can be administered systemically or locally. Such

administration may be parenterally, transmucosally, e.g., orally, nasally, rectally, intravaginally, sublingually, submucosally, transdermally, or by inhalation. Preferably, administration is parenteral, e.g., via intravenous or intraperitoneal injection, and also including, but is not limited to, intra-arterial, intramuscular, intradermal and subcutaneous administration. If the compound described herein (e.g. an inhibitor of C5a activity described herein) or a pharmaceutical composition comprising the compound is administered locally, it can be injected directly into the organ or tissue to be treated.

Pharmaceutical compositions adapted for oral administration may be provided as capsules or tablets; as powders or granules; as solutions, syrups or suspensions (in aqueous or non-aqueous liquids); as edible foams or whips; or as emulsions. Tablets or hard gelatine capsules may comprise lactose, starch or derivatives thereof, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, stearic acid or salts thereof. Soft gelatine capsules may comprise vegetable oils, waxes, fats, semi-solid, or liquid polyols etc. Solutions and syrups may comprise water, polyols and sugars.

An active agent intended for oral administration may be coated with or admixed with a material that delays disintegration and/or absorption of the active agent in the gastrointestinal tract (e.g., glyceryl monostearate or glyceryl distearate may be used). Thus, the sustained release of an active agent may be achieved over many hours and, if necessary, the active agent can be protected from being degraded within the stomach. Pharmaceutical compositions for oral administration may be formulated to facilitate release of an active agent at a particular gastrointestinal location due to specific pH or enzymatic conditions.

Pharmaceutical compositions adapted for transdermal administration may be provided as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Pharmaceutical compositions adapted for topical administration may be provided as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils. For topical administration to the skin, mouth, eye or other external tissues a topical ointment or cream is preferably used. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-water base or a water-in-oil base. Pharmaceutical compositions adapted for topical administration to the eye include eye drops. In these compositions, the active ingredient can be dissolved or suspended in a suitable carrier, e.g., in an aqueous solvent. Pharmaceutical compositions adapted for topical administration in the mouth include lozenges, pastilles and mouthwashes.

Pharmaceutical compositions adapted for nasal administration may comprise solid carriers such as powders (preferably having a particle size in the range of 20 to 500 microns). Powders can be administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nose from a container of powder held close to the nose. Alternatively, compositions
5 adopted for nasal administration may comprise liquid carriers, e.g., nasal sprays or nasal drops. These compositions may comprise aqueous or oil solutions of the active ingredient. Compositions for administration by inhalation may be supplied in specially adapted devices including, but not limited to, pressurized aerosols, nebulizers or insufflators, which can be constructed so as to provide predetermined dosages of the active ingredient. Pharmaceutical
10 compositions may also be administered via the nasal cavity to the lungs.

Pharmaceutical compositions adapted for rectal administration may be provided as suppositories or enemas. Pharmaceutical compositions adapted for vaginal administration may be provided as pessaries, tampons, creams, gels, pastes, foams or spray formulations.

Pharmaceutical compositions adapted for parenteral administration include aqueous and
15 non-aqueous sterile injectable solutions or suspensions, which may contain antioxidants, buffers, bacteriostats and solutes that render the compositions substantially isotonic with the blood of an intended recipient. Other components that may be present in such compositions include water, alcohols, polyols, glycerine and vegetable oils, for example. Compositions adapted for parenteral administration may be presented in unit-dose or multi-dose containers,
20 for example sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of a sterile liquid carrier, e.g., sterile saline solution for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

In a preferred embodiment, a compound described herein (e.g. an inhibitor of C5a
25 activity described herein) is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients
30 are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically-sealed container such as an ampule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an

ampule of sterile saline can be provided so that the ingredients may be mixed prior to administration.

In another embodiment, for example, a compound (e.g. an inhibitor of C5a activity described herein) or a pharmaceutical composition comprising the compound can be delivered in a controlled-release system. For example, the compound may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (see Sefton (1987) *CRC Crit. Ref. Biomed. Eng.* **14**: 201; Buchwald et al. (1980) *Surgery* **88**:507; Saudek et al. (1989) *N. Eng. J. Med.* **321**: 574). In another embodiment, the compound can be delivered in a vesicle, in particular a liposome (see Langer (1990) *Science* **249**:1527-1533; Treat et al. (1989) in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, N.Y., 353-365; WO 91/04014; U.S. 4,704,355). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release* (1974) Langer and Wise (eds.), CRC Press: Boca Raton, Fla.; *Controlled Drug Bioavailability, Drug Product Design and Performance*, (1984) Smolen and Ball (eds.), Wiley: N.Y.; Ranger and Peppas (1953) *J. Macromol. Sci. Rev. Macromol. Chem.* **23**: 61; see also Levy et al. (1985) *Science* **228**:190; During et al. (1989) *Ann. Neurol.* **25**: 351; Howard et al. (1989) *J. Neurosurg.* **71**: 105).

In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the target cells, tissue or organ, thus requiring only a fraction of the systemic dose (see, e.g., Goodson (1984) 115-138 in *Medical Applications of Controlled Release*, vol. 2). Other controlled release systems are discussed in the review by Langer (1990, *Science* **249**: 1527-1533).

In a specific embodiment, it may be desirable to administer a compound described herein (e.g. an inhibitor of C5a activity described herein) or a pharmaceutical composition comprising the compound locally to the area in need of treatment. This may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as silastic membranes, or fibers.

Selection of the preferred effective dose will be determined by a skilled artisan based upon considering several factors which will be known to one of ordinary skill in the art. Such factors include the particular form of the pharmaceutical composition, e.g. polypeptide or vector, and its pharmacokinetic parameters such as bioavailability, metabolism, half-life, etc., which will have been established during the usual development procedures typically employed

in obtaining regulatory approval for a pharmaceutical compound. Further factors in considering the dose include the condition or disease to be prevented and or treated or the benefit to be achieved in a normal individual, the body mass of the patient, the route of administration, whether administration is acute or chronic, concomitant medications, and other factors well known to affect the efficacy of administered pharmaceutical agents. Thus the precise dosage should be decided according to the judgment of the practitioner and each patient's circumstances, e.g., depending upon the condition and the immune status of the individual patient, according to standard clinical techniques.

10

EXAMPLES

The following Examples are provided for further illustration of the invention. The invention, however, is not limited thereto, and the following Examples merely show the practicability of the invention on the basis of the above description.

15 **1. METHODS**1.1 Preparation of Zymosan A stock solution and Zymosan A-activated plasma (ZAP)

Zymosan A was dissolved to 2 mg/ml in 50 ml sterile saline and boiled for 1 h at 100°C. After centrifugation, supernatant was discarded and the pellet was resuspended in 50 ml sterile saline. After a second centrifugation step, pellet was resuspended in 5 ml sterile saline to obtain a 20 mg/ml stock solution. Stock solution was aliquoted and stored at -20°C until use. To activate the plasma, Zymosan A stock solution and 100 µl plasma were mixed and incubated at 37°C for 30 min. After incubation, tubes were centrifuged and the supernatant was aliquoted and stored at -20°C until use.

25 1.2 CD11b assay using rhC5a or ZAP as stimulants

Human whole blood was stimulated with rhC5a or ZAP. To test the blocking activity of IFX-1 and irrelative control IgG4 on rhC5a, the antibodies were diluted to final Ab/Ag molar ratios of 1:1 and 0.5:1. To test the blocking activity of IFX-1 on eC5a, IFX-1 was diluted to reach final Ab/Ag molar ratios of approximately 4:1/3:1/2:1/1:1/0.5:1. Blood only with buffer served as a non-stimulation control to assess the baseline CD11b expression. Blood with antibody alone was used to determine the effects on CD11b expression of the antibody under non-stimulated condition. The complete mixture (Ab/Ag/blood) was incubated at 37°C for 20 min to evaluate C5a-induced up-regulation of CD11b. After addition of anti-mouse CD11b:FITC samples were incubated for 30 min on ice to minimize background staining.

Granulocytes were gated and mean fluorescence intensity (MFI) of FITC labeled (CD11b expressing) granulocytes was examined by flow cytometer.

1.3 CD11b assay using rhC5a or zymosan A in the whole blood

5 Human blood was stimulated with rhC5a or zymosan A, and the complete mixture (Ab/Ag/blood) was incubated at 37°C for 20 min to stimulate the C5a-induced up-regulation of CD11b. After incubation, 2 µl of anti-mouse CD11b:FITC or isotype:FITC control was added and samples were incubated for 30 min on ice to minimize background staining. After lysis, cells were analyzed using flow cytometer. On the FSC/SSC dot-plot, granulocytes were gated
10 and mean fluorescence intensity (MFI) of FITC labeled (CD11b expressing) granulocytes was examined for the whole sample set.

1.4 Cytokine IL-8 ELISA

Human IL-8 ELISA was performed as recommended in the instruction manual under
15 section “Assay procedure” (eBioscience Inc., San Diego, CA). Briefly, coating was performed overnight at 4°C using 100 µl 1x capture antibody. Plates were blocked using 200 µl 1x assay diluents at RT for 1 h. Standard stock solutions were diluted with 1x assay diluents to the desired concentration, followed by 6 serial 1:2 dilutions. Sample supernatants were diluted as required in 1x assay diluents. According to the “Assay procedure”, 100 µl of standard dilutions and
20 sample dilutions were added to the coated plate and incubated at RT for 1 h, followed by the incubation with 100 µl 1x detection antibody (RT, 1 h) and 100 µl 1x avidin-HRP (RT, 30 min). Color development was performed with 100 µl TMB substrate solution at RT for 10 min in the dark and was terminated with 100 µl stop solution. Absorbance was read out within 30 min using the plate reader at 450 nm. Zero standard value (blank) was subtracted from all standards
25 and samples. Cytokine concentration of samples was calculated using a $\log(x) / \log(y)$ standard curve of included standard samples.

1.5 C5a ELISA

Purified anti-human C5a monoclonal antibody (InflaRx GmbH, Jena, Germany) was
30 coated overnight with a final concentration of 0.5 µg/mL on the ELISA plate. After blocking with the assay diluent (1x PBS with 0.05% Tween 20 and 2% heat-inactivated FBS), calibration samples (recombinant human C5a, Sigma, Taufkirchen, Germany) and samples diluted in assay diluent were incubated for 90 minutes at room temperature. Mouse anti-human C5/C5a antibody clone 561 (Hycult Biotech, Uden, The Netherlands) diluted to 2 µg/mL in assay

diluent was applied as the primary detection antibody for a 60-minute incubation at room temperature, followed by a 30-minute incubation with the secondary horseradish peroxidase labeled antibody (goat anti-mouse IgG2a polyclonal antibody, SouthernBiotech, Birmingham, USA) diluted to 0.05 µg/mL in assay diluent. Color development was performed with tetramethylbenzidine substrate solution (TMB, Biozol, Eching, Germany) and was stopped with 3.7 N sulfuric acid. The OD was read as the absorbance of 450 nm by Tecan Infinite® 200 reader with Tecan Magellan™ (Tecan Group, Maennedorf, Switzerland). The in-house developed C5a ELISA was validated according to the EMA guideline on bioanalytical method validation.

Intra-assay and inter-assay precision tested with five different concentrations showed a coefficient of variance (CV) of 0.65 % to 4.96 % and 1.50 % to 4.88 % for six and 18 repetitions, respectively. Recovery analysis of the spiked recombinant human C5a in buffer resulted in recoveries of 86.98 ± 1.20 % (mean $\pm$ SD) at the lower limit of quantification and 91.50 ± 3.29 % at the upper limit of quantification. No cross-reactivity for C3, C3a and C4 and cross-reactivity of $< 0.01\%$ for C5b-6 was detected. Human IgG4 antibodies did not interfere with the assay. The mean C5a level in citrate plasma from 20 human volunteers is 17.08 ng/mL $\pm$ 6.96 ng/mL with a range from 7.52 ng/mL to 30.17 ng/mL.

1.6 Measurements of complement activation products

Concentrations of complement activation products C3a, C5a and membrane attack complex C5b-9 were measured by ELISA. C3a ELISA (BD OptEIA™ Human C3a ELISA Kit, BD Bioscience, Germany) was conducted according to the manufacturer instruction. C5b-9 concentration was determined using the C5b-9 ELISA validated by InflaRx based on the BD OptEIA™ Human C5b-9 ELISA Set (BD Bioscience). C5a concentration was measured using the C5a ELISA established and validated by InflaRx described above.

1.7 Statistical analysis

All results were expressed as the mean $\pm$ standard deviation. Statistical differences between groups, after baseline correction, were calculated by One-Way-ANOVA, including Tukey's multiple comparison test or by the students t-test for two groups. The p value of 0.05 was used in the calculation to determine whether there were any significant differences between any two groups. Creating of graphs and statistical analysis were performed with GraphPad PRISM® V6.05 (CA, USA).

2. PRECLINICAL RELEVANT DATA

2.1 Neutrophils activation by C5a and the blocking effect of IFX-1

As CD11b up-regulation is a sensitive hallmark for neutrophil activation, CD11b levels on neutrophils were employed to evaluate the neutrophil activation. The human whole blood model was used to assess the blocking activity of IFX-1 to recombinant human C5a (rhC5a) in this study. Human whole blood was incubated with buffer, antibody alone, rhC5a alone, or combinations of different antibody concentration and rhC5a. After incubation, cells were stained with anti- mouse CD11b:FITC and CD11b MFI was analysed by flow cytometry checking for the activation levels of blood neutrophils. As shown in **Figure 1**, recombinant human C5a strongly stimulates the CD11b up-regulation on human neutrophils. This effect can be completely blocked in presence of the anti-human C5a antibody IFX-1. This inhibition is highly specific and the irrelevant human IgG4 antibody did not show any blocking activity.

As a source for endogenous C5a (eC5a), zymosan-activated plasma (ZAP) was used to stimulate the blood neutrophils. The amount of eC5a in ZAP was measured using a commercial C5a ELISA Kit. The data presented here (**Figure 2**) point out that eC5a in ZAP induced comparable levels of CD11b up-regulation to rhC5a. The presence of IFX-1 significantly decreased the CD11b expression on human neutrophils, even at an Ab:Ag molar ratio of 0.5:1. The overall blocking activity of IFX-1 to ZAP-induced CD11b up-regulation ranged from 100% to 82% depending on the Ab:Ag ratio. Despite the presence of high levels of eC3a and other complement activation products in ZAP, IFX-1 could specifically block CD11b upregulation up to 100%. It can therefore be concluded that eC5a is the sole driver for neutrophil activation upon ZAP stimulation, and IFX-1 can completely block it.

2.2 C5a blockade attenuates zymosan-induced inflammatory responses in the human whole blood

Zymosan A, as an active fungus cell wall component, can induce strong inflammatory responses in human whole blood as characterized by the activation of neutrophils with elevated cytokines and chemokines levels. In this study, human whole blood was spiked with zymosan A in the presence or absence of IFX-1, and CD11b expression on blood neutrophils was measured by flow cytometric analysis. As shown in **Figure 3**, CD11b on blood neutrophils was strongly upregulated at the presence of zymosan in human whole blood. The zymosan-stimulated CD11b upregulation can be suppressed by 79% – 93% depending on the concentration of IFX-1 added. As a positive control, the CD11b up-regulation stimulated by rhC5a was 100% blocked by IFX-1. Therefore, it is affirmative that the CD11b up-regulation

on blood neutrophils upon zymosan A stimulation is caused primarily by eC5a. In addition, it can be concluded that eC5a, once generated in the whole blood by zymosan A, binds to IFX-1 first, thereby blocking its access to its natural receptors.

In the same experimental set-up, IL-8 levels were measured and used to assess the inflammatory response. IL-8 concentrations after various doses of zymosan A stimulation ranged from 458 pg/ml to 3218 pg/ml in the absence of IFX-1. As shown in Figure 4, the presence of IFX-1 significantly reduced IL-8 generation upon stimulation with various concentrations of zymosan A and the reduction rate up to 54% was observed. Thus, in the whole blood setting of inflammation, zymosan-induced inflammatory responses are largely dependent on the presence of C5a.

3. CLINICAL RELEVANT DATA

3.1 DATA OBTAINED FROM CLINICAL SAMPLES

3.1.1 Complement activation in HS patients

A total of 54 patients with HS and 14 healthy volunteers were enrolled in the study. Patients are under follow-up in the Outpatient Department of Immunology of Infectious Diseases of the ATTIKON University Hospital, Greece. The study was approved by the Ethics Committee of the hospital. Written informed consent was provided by all patients. Diagnosis of HS was based on the following criteria: a) onset early after puberty; b) presence of subcutaneous nodules in areas of skin rich in apocrine glands; and c) a compatible history of recurrent drainage of pus from the affected areas.

Circulating concentrations of complement factors C3a and C5a as well as membrane attack complex sC5b-9 were determined in the plasma of 54 patients and of 14 healthy controls as well as in the pus of seven patients. As shown in Figure 5, circulating C5a was significantly greater in patient plasma than in control plasma ($P < 0.01$), and the differences of C3a and C5b-9 between patients and controls were of similar significance. Therefore, it can be concluded that systemic complement activation occurs in HS. Given the essential role of complement activation in the innate and adaptive immunity, the inventors assumed that targeting of complement activation could be a new therapeutic strategy for the treatment of HS.

However, from the above results it was not clear which one of C3a, C5a or C5b-9 or other complement activation products would be the most promising target for this new therapeutic strategy and whether it would be sufficient to target only one of these factors or whether two or more factors involved in complement activation have to be targeted.

3.1.2 Blocking of CD11b upregulation on blood neutrophils induced by HS plasma

To determine the role of C5a in the HS plasma sample on the neutrophil activation, the HS plasma samples with high levels of C5a were chosen and assessed by employing the human whole blood model. As shown in Figure 6, in contrast to the control plasma samples with low C5a levels (Ctrl 008 and Ctrl 012), HS plasma samples (Pat. 088 and Pat. 092) with high levels of C5a strongly upregulated CD11b expression on blood neutrophils. Recombinant human C5a was used as the positive control, while the plasma from healthy volunteers was chosen as the negative control. The CD11b upregulation induced by HS plasma can be 100% suppressed by IFX-1, indicating that C5a is the most important activator in the HS plasma to initiate neutrophil activation. From these novel results the inventors concluded that blockade of C5a in HS patients is sufficient to achieve a strong suppression on neutrophil activation.

3.2 DATA OBTAINED FROM CLINICAL TRIAL

3.2.1 Trial Design

An open label Phase II trial in 11 patients with moderate to severe hidradenitis suppurativa was conducted in Department of Internal Medicine, ATTIKON University Hospital, Greece.

Primary objective of the trial was to explore the safety and tolerability of IFX-1 administered over 8 weeks. Secondary objectives of the trial were to assess the pharmacokinetics and pharmacodynamics of IFX-1 as well as to generate preliminary data on the efficacy of IFX-1 on clinical endpoints (e.g., HiSCR, DLQI, VAS for disease status, VAS for pain, HS-PGA, modified Sartorius Score) to generate further hypotheses. The enrolled patients were treated with 800 mg IFX-1 twice in the first week and once a week thereafter for the total 8-week treatment; i.e. IFX-1 was administered in nine intravenous doses of 800 mg IFX-1 on days 1, 4, 8, 15, 22, 29, 36, 43, and 50. All patients were followed up for 12 additional weeks.

Inclusion Criteria at Screening:

1. Male or female patients ≥ 18 years old
2. Written informed consent
3. Diagnosis of HS for at least 1 year
4. HS lesions in at least 2 distinct anatomic areas, one of which is Hurley Stage II or III
5. Total AN (abscesses and nodules) count ≥ 3

6. Patients with either primary or secondary failure of biological treatment or are not eligible for treatment with other biologicals

NOTE: a primary failure is defined as an at least 12 week treatment with a biological compound without effect and a secondary failure as achieving an initial response after at least 12 week treatment with a biological compound followed by a relapse.

7. Failure of previous antimicrobial treatments

Exclusion Criteria at Screening:

1. Body weight above 150 kg or body weight below 60 kg
2. Has a draining fistula count of greater than 30 at baseline
3. Surgical management planned within the next 24 weeks
4. Occurrence of a flare-up of HS leading to intravenous antimicrobial treatment within the last 14 days
5. Any other disease and condition that is likely to interfere with evaluation of study product, outcome assessment or satisfactory conduct of the study
 - a) Active infection
 - b) Severe congestive heart failure (i.e., NYHA Class IV)
 - c) Depression
 - d) History of systemic lupus erythematosus or rheumatoid arthritis
 - e) Any immunodeficiency disease
 - f) Active hematological or solid malignant tumor
 - g) Patients must not have had any other active skin disease or condition (e.g., bacterial, fungal, or viral infection) that may have interfered with assessment of HS.
6. One of the following abnormal laboratory results
 - a) White blood cell count $< 2,500/\text{mm}^3$
 - b) Neutrophil count $< 1000/\text{mm}^3$
 - c) Serum creatinine $> 3 \times$ Upper Normal Limit (UNL)
 - d) Total bilirubin $> 2 \times$ UNL
 - e) Alanine-Aminotransferase (ALAT) $> 2 \times$ UNL
 - f) Positive screening test for Hepatitis B, Hepatitis C, or HIV 1/2
7. Prior administration of any biological compound in the last 3 months
8. Intake of corticosteroids defined as daily intake of prednisone or equivalent more than 1 mg/kg for the last three weeks;

9. Intake of immunosuppressive drugs within the past 30 days (e.g., cyclosporine, tacrolimus)

10. General exclusion criteria

a) Pregnant (in women of childbearing potential an urine pregnancy test has to be performed) or breast-feeding women

b) Women with childbearing potential (defined as within two years of their last menstruation) not willing to practice appropriate contraceptive measures (e.g., implanon, injections, oral contraceptives, intrauterine devices, partner with vasectomy, abstinence) while participating in the trial

c) Participation in any interventional clinical trial within the last three months

d) Known intravenous drug abuse

e) Employee at the study site, spouse/partner or relative of any study staff (e.g., investigator, sub-investigators, or study nurse) or relationship to the sponsor

3.2.2 Clinical Trial Findings

IFX-1 is well tolerated by HS patients. There were no drug-related serious adverse events reported over the treatment period.

A commonly used efficacy parameter in the Hidradenitis Suppurativa Clinical Response (HiSCR). HiSCR is defined by the status of three types of lesions (defining criteria): abscesses (fluctuant, with or without drainage, tender or painful), inflammatory nodules (tender, erythematous, pyogenic granuloma lesion) and draining fistulas (sinus tracts, with communications to skin surface, draining purulent fluid). The proposed definition of responders to treatment (HiSCR achievers) is: (i) at least a 50% reduction in ANs, (ii) no increase in the number of abscesses, and (iii) no increase in the number of draining fistulas from baseline.

HiSCR has been validated recently as a responsive and clinically meaningful endpoint of the inflammatory manifestation of HS (Kimball and others, 2014).

The HiSCR response over the treatment period of 8 weeks was investigated in this study, and 8 out of 11 patients already treated up to Day 56 responded, which represents a response rate of 72.7% and a 95% confidence interval of 43% to 91%. To compare these results with historical data a literature search was performed to detect placebo controlled clinical studies that used HiSCR as an efficacy parameter. The following Table 4 summarizes the five studies that were completed recently:

Table 4. Completed clinical studies using HiSCR as an efficacy parameter.

Compound	N	Placebo responder n (%)	Comment
Adalimumab ¹	13	2 (15%)	Post hoc analysis of Phase II trial. Only subgroup of patients with Hurley III
Adalimumab ¹	70	15 (21%)	Study313, subgroup of patients with Hurley III
Adalimumab ¹	76	13 (17%)	Study810, subgroup of patients with Hurley III
Anakinra ²	10	3 (30%)	All patients. 6 of 10 patients had Hurley III
MABp1 ³	10	1 (10%)	Anti-TNFα treatment failures

¹ Humira EMA assessment report:

http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000481/WC500195564.pdf

5 ² Anakinra Study (Tzanetakou and others, 2016).

³ Press release XBiotech (<http://investors.xbiotech.com/phoenix.zhtml?c=253990&p=irol-newsArticle&ID=2246777>)

10 In total, 179 patients have been treated in the placebo group of these studies with a response rate of 19.0% with a 95%-confidence interval of 14% to 25%. As both confidence intervals (e.g., the historical placebo patients and the patients treated with IFX-1) are not overlapping, a significant treatment effect of IFX-1 can be concluded.

15 Photographic documentation of the affected areas confirmed these findings by a highly reduced inflammation on the skin, as evidenced by the visual reduction of inflammatory swollenness and redness post treatment.

Thus, anti-C5a represents a powerful anti-inflammatory agent in the disease setting of HS. This clinical finding demonstrates that blockade of C5a is highly effective to reduce the activation of neutrophils thereby effectively alleviating cutaneous neutrophilic inflammatory disorders.

4. BLOCKING THE CD11B UPREGULATION INDUCED BY ACTIVATED COMPLEMENT FACTOR IN *HIDRADENITIS SUPPURATIVA* PATIENTS VIA C5A-C5AR AXIS INHIBITION

4.1 PURPOSE

5 The purpose of the following study was to demonstrate the blockade of *Hidradenitis suppurativa* (HS) patient plasma-induced CD11b upregulation on the surface of neutrophils by anti-human C5a monoclonal antibody IFX-1, anti-human C5a receptor C5aR (CD88) antibodies, and a C5aR antagonist as well as a C5aR inhibitor.

10 4.2 ASSAY PRINCIPLE

Accumulation of neutrophils at the site of inflammation is dependent on the expression of adhesion molecules, including CD11b (also known as integrin alpha M) (Larson and Springer, 1990; Carlos and Harlan, 1990). Upregulation and mobilizing of CD11b/CD18 from intracellular pools to the surface of neutrophils is essential for the rolling action and migration
15 of human neutrophils (Smith et al., 1989). Enhanced expression of CD11b/CD18 therefore reflects an inflammatory triggering event. The human CD11b assay is conducted using flow cytometry to detect FITC-conjugated anti-CD11b antibody on the surface of neutrophils. Activated complement products, especially elevated endogenous C5a (eC5a) in HS patient plasma samples can strongly upregulate CD11b expression through the binding of C5a to its
20 receptor C5aR (CD88) on neutrophils. Consequently, blockade of the C5a-C5aR axis is expected to abolish or attenuate CD11b upregulation on the surface of neutrophils.

As the first anti-human C5a monoclonal antibody introduced into clinical development IFX-1 has been demonstrated to control the inflammatory responses that lead to tissue and organ damage. This antibody is currently being evaluated in a Phase IIb study for patients with
25 moderate or severe *Hidradenitis suppurativa*. It specifically and directly neutralizes the terminal complement anaphylatoxin C5a and blocks its harmful effects as the key inflammatory mediator in both acute and chronic inflammatory diseases (Klos et al., 2009; Guo and Ward, 2005; Riedemann et al., 2017).

C5a exerts its effects through interacting with the high-affinity C5a receptors (C5aR and
30 C5L2) (Guo and Ward, 2005). C5aR belongs to the rhodopsin family of G-protein-coupled receptors with seven transmembrane segments, while C5L2 is not G-protein-coupled. It is generally understood that C5a-C5aR signaling is very important in the pathogenesis of proinflammatory outcomes (Ward, 2009). Therefore, targeting the C5aR is another strategy for inhibiting complement-dependent inflammatory diseases. A series of small molecules derived

from the C-terminus of C5a were developed as C5aR antagonists. Among them, the lead compound cyclic hexapeptide PMX-53 (AcF-[OP(D-Cha)WR]) (Finch et al., 1999) was shown to attenuate injury in numerous animal models of inflammation following intravenous, subcutaneous, intraperitoneal, and oral administration (Proctor et al. 2006). With their structural similarity to C5a, such antagonists compete with C5a for the C5a receptors on neutrophils (March et al., 2004). In addition, anti-C5aR antibodies could block the binding of C5a to C5aR, thereby reducing the accumulation and activation of myeloid-derived suppressor cells and neutrophils (Markiewski et al., 2008). Two commercially available monoclonal anti-C5aR antibodies were tested in this study, clones S5/1 and 7H110. They were raised in mouse against a synthetic peptide comprising the N-terminal extracellular domain of C5aR (Met1-Asn31) and the recombinant human C5aR (Met1-Val350), respectively, and both antibodies were described as neutralizing antibodies. Avacopan (CCX168) is an orally-administered small molecule drug candidate that selectively inhibits the complement C5a receptor (C5aR), and is being developed for inflammatory and autoimmune diseases (Bekker et al., 2008; Jayne et al., 2017)

The inhibitory effect of these blocking agents that target the C5a-C5aR axis was monitored via flow cytometry for the blockade of CD11b upregulation on neutrophils.

4.3 EXPERIMENTAL DETAILS

4.3.1 Samples

According to the consensus definition and diagnostic criteria of the Hidradenitis Suppurativa Foundation 2009, plasma samples of two HS patients (Pat. 088 & Pat. 092) and two healthy controls (Ctrl 009 & Ctrl 010) were included in this study. The complement system was activated in the pathogenesis of HS as manifested by the elevated levels of C3a, C5a and C5b-9 (see Table 5 below). The C5a levels of patients 088 and 092 (93.77 ng/mL and 70.01 ng/mL, respectively) are significantly higher than those of healthy controls (21.02 ng/mL and 11.77 ng/mL).

Table 5. Concentrations of complement factors measured in plasma samples of the study objects

Patient ID	Matrix	C5a [ng/ml]	C3a [ng/ml]	C5b-9 [ng/ml]
Pat. 088	plasma	93.77	13177.50	>max
Pat. 092	plasma	70.01	8801.00	276.12
Ctrl 009	plasma	21.02	1818.68	122.76
Ctrl 010	plasma	11.77	2625.40	129.52

4.3.2 Reagents

- Analara water, VWR (Darmstadt, Germany), Cat. No. 102923C, NORMAPUR for analysis, sterile filtered
- 5 • ACD, Sigma Aldrich (Taufkirchen, Germany), Cat. No. C3821-50ML
- Reagents for flow cytometer
 - FACS Flow Sheat Fluid, BD Bioscience (NJ, USA), Cat. No. 342003
 - FACS Shutdown solution, BD Bioscience (NJ, USA), Cat. No. 334224
 - FACS Clean solution, BD Bioscience (NJ, USA), Cat. No. 340345
 - 10 ○ rat anti-mouse CD11b:FITC, BD Bioscience (NJ, USA) Cat. No. 553310, 0.5 mg/mL
 - 10× FACS Lysing solution, BD Bioscience (NJ, USA), Cat. No. 349202 → Working solution: 1× FACS Lysing solution (1:10 diluted in Analara water)
 - Staining buffer: 1% heat-inactivated FBS + 0.1% sodium azide in 1× PBS solution
 - 15 • FBS, Thermo Fisher Scientific (Darmstadt, Germany), Cat. No. 10099133
heat-inactivation: 56°C, 30 min
 - PBS powder, Sigma Aldrich (Taufkirchen, Germany), Cat. No. P3813-10PAK
 - sodium azide, VWR (Darmstadt, Germany), Cat. No. 1.06688.0250
 - recombinant human C5a (rhC5a), Hycult Biotech (Uden, Netherlands), Cat. No. HC2101,
20 expressed in *E. coli*, dissolved in sterile Analara water
 - 0.9% sterile sodium chloride (saline), B.Braun (Melsungen, Germany), Cat. No. 3200950
 - IFX-1, anti-human C5a antibody applied as control, InflaRx (Jena, Germany), 10 mg/mL in PBS + 0.05% Tween80
 - PMX-53, bio-technie (Wiesbaden-Nordenstadt, Germany), Cat. No. 5473
 - 25 • anti-C5aR (CD88) antibody Clone S5/1, Hycult Biotech (Uden, Netherlands), Cat. No. HM2094
 - anti-C5aR (CD88) antibody Clone 7H110, biomol (Hamburg, Germany), Cat. No. C2439-60N
 - Avacopan, MedKoo Biosciences Inc. (Morrisville, USA), Cat. No. 319575
 - 30 • human blood (immediate use) from healthy donor containing 12% ACD
 - human plasma pool (citrate plasma) from Jena University Hospital.

4.3.3 Equipment

- Flow Cytometer (FACS Canto II with DIVA software V6.1.2)

4.3.4 Procedures

a) Human CD11b Potency Assay (Flow Cytometric Assay)

Two patient plasma samples Pat. 088 and Pat. 092 (5 μ L) were incubated with fresh human blood (60 μ L, as source of neutrophils) in the absence or presence of C5a-C5aR axis blockers (anti-C5a antibody IFX-1, anti-C5aR antibodies clone S5/1 and clone 7H110, C5aR antagonist PMX-53, and C5aR inhibitor Avacopan; 10 μ L) in a total volume of 100 μ L. Two control plasma samples (Ctrl 009 and Ctrl 010, 5 μ L) prepared according to the procedure applied to the patient samples served as controls for unspecific activation. Blood with only saline (40 μ L) or normal human plasma pool (huPP 5 μ L + saline 35 μ L) served as the non-stimulated control to define the baseline expression of CD11b. Blood sample with normal human plasma pool and spiked with recombinant human C5a (rhC5a) mimicked the stimulated condition. All samples were incubated at 37°C for 20 min to activate CD11b upregulation. After cooling on ice, 2 μ L of FITC-conjugated anti-mouse CD11b antibody was added to the samples. The labeled samples were kept on ice in the dark for another 30-min to minimize the background signal. Red blood cells were then lysed with 1 $\times$ FACS Lysing solution at room temperature for 10 min. 2 mL Staining buffer was used to wash the remaining cells twice. After centrifugation at 2500 rpm for 3 min, cells were resuspended in 0.5 mL Staining buffer and ready for FACS analysis. A gate was set on the FSC vs. SSC plot to allow analysis only of cells with the size of neutrophils.

The percentage of blocking activity (BA) of the C5a-C5aR axis blockade test material was calculated using the formula below. Where MFI is the mean fluorescence intensity emitted from the CD11b-bound FITC on neutrophils.

$$BA (\%) = (MFI_{\text{Patient plasma}} - MFI_{\text{Test material spiked patient plasma}}) \div (MFI_{\text{Patient plasma}} - MFI_{\text{huPP}}) \times 100$$

b) Statistical analysis

Graphs were created with GraphPad Prism 7 (CA, USA).

4.4 RESULTS AND DISCUSSIONS

Expression of Integrin CD11b on Neutrophils

CD11b expression on neutrophils was evaluated with plasma samples from two healthy blood donors (Ctrl 009 and Ctrl 010) and two diagnosed HS patients (Pat. 088 and Pat. 092). The mean fluorescence intensity (MFI) of the Ctrl-samples was 2156.9 ± 114.3 , which falls within the non-stimulated CD11b baseline expression range ($MFI \leq 3500$). In contrast, a 2.3 to

3.9-fold elevation of CD11b expression was induced either by HS patient samples (Pat. 088, Pat. 092) or by 20 nM recombinant human C5a (rhC5a) (Figures 8, 9, 10 and 11; Tables 6, 7 and 8). These data suggest that the significantly upregulated CD11b expression was mediated by the inflammatory factors in HS patients. It is postulated that the complement activation products, especially C5a, play a major role in the CD11b upregulation (Table 5).

Table 6. Blocking activity of anti-C5aR antibodies clones S5/1 and 7H110, and C5aR antagonist PMX-53 on complement factor-induced CD11b upregulation.

ID	Description	CD11b (mean MFI)	Blocking activity (%)
A	Saline	2049.5	
B1	huPP	2166.5	
B2	huPP Clone S5/1 (50 nM)	2247	
B3	huPP Clone 7H110 (50 nM)	2401	
B4	huPP PMX 53 (50 nM)	2053.5	
D3	huPP rhC5a (20 nM)	6329.5	—
E7	huPP rhC5a Clone S5/1 (50 nM)	3288.5	73.05
E8	huPP rhC5a Clone 7H110 (50 nM)	3385	70.73
E9	huPP rhC5a PMX 53 (50 nM)	4235.5	50.30
C1	Ctrl 010	2102.5	
C2	Ctrl 009	2018.5	
D1	Pat. 088	7918	—
E1	Pat. 088 Clone S5/1 (50 nM)	3736.5	72.70
E2	Pat. 088 Clone 7H110 (50 nM)	3564.5	75.69
E3	Pat. 088 PMX 53 (50 nM)	4728.5	55.46
D2	Pat. 092	7206.5	—
E4	Pat. 092 Clone S5/1 (50 nM)	3555	75.86
E5	Pat. 092 Clone 7H110 (50 nM)	3393.5	78.67
E6	Pat. 092 PMX 53 (50 nM)	4657.5	56.69

Table 7. Blocking activity of anti-C5a antibody IFX-1 and C5aR antagonist PMX-53 on complement factor-induced CD11b upregulation.

ID	Description	CD11b (mean MFI)	Blocking activity (%)
A	Saline	2516.5	
B1	huPP	2324.5	
B2	huPP PMX 53 (20 μ M)	2218.5	
B3	huPP IFX-1 (20 nM)	2352	
D3	huPP rhC5a (20 nM)	8332.5	—
E5	huPP rhC5a PMX 53 (20 μ M)	1851	107.88
E6	huPP rhC5a IFX-1 (20 nM)	2067	104.29
C1	Ctrl 010	2241	
C2	Ctrl 009	2265.5	
D1	Pat. 088	8955	—
E1	Pat. 088 PMX 53 (20 μ M)	2027.5	104.48
E2	Pat. 088 IFX-1 (20 nM)	2209	101.74
D2	Pat. 092	7120.5	—
E3	Pat. 092 PMX 53 (20 μ M)	1912.5	108.59
E4	Pat. 092 IFX-1 (20 nM)	2147	103.70

5 Inhibition of CD11b upregulation by anti-human C5aR monoclonal antibodies, C5aR antagonist and C5aR inhibitor

As shown in Figure 8, rhC5a (20 nM) and two HS patient plasma samples with high levels of eC5a (Pat. 088 and Pat. 092) strongly upregulated CD11b expression on blood neutrophils, while the presence of blocking antibodies targeting C5aR with a final concentration of 50 nM could significantly attenuate CD11b expression driven by either rhC5a or HS patient plasma samples. High blocking activities ranging from 71% to 79% were achieved by using anti-C5aR antibodies, clone 7H110 and clone S5/1 (Figure 8, Tables 9 and 6). In contrast, the C5aR antagonist PMX-53, which is a small hexapeptide, was not as effective as the C5aR-specific monoclonal antibodies. The blocking activities rendered by the same concentration of PMX-53 (50 nM) were within 50% to 57% (Figure 9A, Tables 9 and 6). To examine whether abolishment of CD11b upregulation can be achieved *via* C5aR inhibition, a high concentration of PMX-53 (20 μ M) was further evaluated for the blocking activities under the same experimental set-up. As a result, the CD11b upregulation induced by HS patient plasma samples

as well as by rhC5a was completely abrogated in the presence of high levels of PMX-53 (Figure 9B, Tables 9 and 7). Similar data were achieved in the presence of Avacopan, a C5aR inhibitor (Figure 11, Tables 8 and 9). The blocking activities in the presence of 100 μ M Avacopan were within 77% to 80%, whereas the CD11b upregulation induced by HS patient plasma was completely blocked in the presence of 500 μ M Avacopan (Figure 11, Tables 8 and 9).

These results suggested that the C5a/C5aR axis is predominantly responsible for the CD11b upregulation on blood neutrophils, and that C5aR can serve as a potential target for blocking C5a activities.

Table 8. Blocking activity of C5aR inhibitor Avacopan on complement factor-induced CD11b upregulation.

ID	Description	CD11b (mean MFI)	Blocking activity (%)
A	Saline	3767	
B1	huPP	3407	
B2	Ctrl 010	3208.5	
B3	Ctrl 009	3125.5	
C1	Pat. 088	11717.5	–
D1	Pat. 088 Avacopan (500 μ M)	3027	104.57
D2	Pat. 088 Avacopan (100 μ M)	5289	77.35
C2	Pat. 092	11214.5	–
D3	Pat. 092 Avacopan (500 μ M)	3081.5	104.17
D4	Pat. 092 Avacopan (100 μ M)	5000	79.60

Table 9. Summarized blocking activities (%) of all C5a-C5aR axis blockade test molecules on CD11b upregulation induced by HS patient plasma- or rhC5a-spiked healthy human plasma.

Test Material	Target	Blocking Activity (%)	
IFX-1 20 nM	C5a	Pat. 088	101.74
		Pat. 092	103.70
		rhC5a-spiked huPP	104.29
Clone S5/1 50 nM	C5aR	Pat. 088	72.70
		Pat. 092	75.86
		rhC5a-spiked huPP	73.05
Clone 7H110 50 nM	C5aR	Pat. 088	75.69
		Pat. 092	78.67
		rhC5a-spiked huPP	70.73
PMX-53 50 nM	C5aR	Pat. 088	55.46
		Pat. 092	56.69
		rhC5a-spiked huPP	50.30
PMX-53 20 μM	C5aR	Pat. 088	104.48
		Pat. 092	108.59
		rhC5a-spiked huPP	107.88
Avacopan 100 μM	C5aR	Pat. 088	77.35
		Pat. 092	79.60
Avacopan 500 μM	C5aR	Pat. 088	104.57
		Pat. 092	104.17

5 IFX-1, the anti-human C5a monoclonal antibody, blocked the HS-induced CD11b upregulation completely

By employing the same experimental set-up described above, as would be expected, rhC5a and HS patient plasma samples (Pat. 088 and Pat. 092) strongly activated CD11b expression on blood neutrophils, and addition of IFX-1 with a concentration as low as 20 nM could completely abolish the CD11b upregulation (Figure 10). These results indicate that IFX-1 is more efficient in inhibiting C5a/C5aR-driven inflammatory responses.

4.5 CONCLUSION

Taken together, our results show that the C5a-targeted approach, *i.e.* the application of anti-C5a monoclonal antibody IFX-1 abolishes C5a-mediated CD11b upregulation effectively. Furthermore, the anti-C5aR antibodies, the C5aR antagonist as well as the C5aR inhibitor also exhibited a strong blocking activity under the same experimental conditions. Thus, targeting C5aR with blocking antibodies or antagonists represents an alternative strategy to block C5a/C5aR axis under inflammatory conditions such as HS.

REFERENCES

- Abi Abdallah DS, Egan CE, Butcher BA, Denkers EY. 2011. Mouse neutrophils are professional antigen-presenting cells programmed to instruct Th1 and Th17 T-cell differentiation. *Int Immunol* 23(5):317-326.
- Bekker, P. et al. Characterization of Pharmacologic and Pharmacokinetic Properties of CCX168, a Potent and Selective Orally Administered Complement 5a Receptor Inhibitor, Based on Preclinical Evaluation and Randomized Phase 1 Clinical Study. *PLoS One* 11, e0164646, doi:10.1371/journal.pone.0164646 (2016).
- Braun-Falco M, Kovnerystyy O, Lohse P, Ruzicka T. 2012. Pyoderma gangrenosum, acne, and suppurative hidradenitis (PASH)--a new autoinflammatory syndrome distinct from PAPA syndrome. *J Am Acad Dermatol* 66(3):409-415.
- Carlos, T. M. & Harlan, J. M. Membrane proteins involved in phagocyte adherence to endothelium. *Immunol Rev* 114, 5-28 (1990).
- Cugno M, Borghi A, Marzano AV. 2017. PAPA, PASH and PAPASH Syndromes: Pathophysiology, Presentation and Treatment. *Am J Clin Dermatol*.
- Cugno M, Gualtierotti R, Meroni PL, Marzano AV. 2018. Inflammatory Joint Disorders and Neutrophilic Dermatoses: a Comprehensive Review. *Clinic Rev Allerg Immunol* 54:269-281, doi:10.1007/s12016-017-8629-0
- Czermak BJ, Sarma V, Pierson CL, Warner RL, Huber-Lang M, Bless NM, Schmal H, Friedl HP, Ward PA. 1999. Protective effects of C5a blockade in sepsis. *Nat Med* 5(7):788-792.
- Finch, A. M. *et al.* Low-molecular-weight peptidic and cyclic antagonists of the receptor for the complement factor C5a. *J Med Chem* 42, 1965-1974, doi:10.1021/jm9806594 (1999)
- Guo RF, Riedemann NC, Sun L, Gao H, Shi KX, Reuben JS, Sarma VJ, Zetoune FS, Ward PA. 2006. Divergent signaling pathways in phagocytic cells during sepsis. *J Immunol* 177(2):1306-1313.
- Guo RF, Ward PA. 2005. Role of C5a in inflammatory responses. *Annu Rev Immunol* 23:821-852, doi:10.1146/annurev.immunol.23.021704.115835 (2005).
- Huber-Lang MS, Sarma JV, McGuire SR, Lu KT, Guo RF, Padgaonkar VA, Younkin EM, Laudes IJ, Riedemann NC, Younger JG and others. 2001. Protective effects of anti-C5a peptide antibodies in experimental sepsis. *FASEB J* 15(3):568-570.
- Huber-Lang MS, Younkin EM, Sarma JV, McGuire SR, Lu KT, Guo RF, Padgaonkar VA, Curnutte JT, Erickson R, Ward PA. 2002. Complement-induced impairment of innate immunity during sepsis. *J Immunol* 169(6):3223-3231.
- Jayne, D. R. W. et al. Randomized Trial of C5a Receptor Inhibitor Avacopan in ANCA-Associated Vasculitis. *J Am Soc Nephrol* 28, 2756-2767, doi:10.1681/ASN.2016111179 (2017).

- Jemec GB. 2004. Medical treatment of hidradenitis suppurativa. *Expert Opin Pharmacother* 5(8):1767-1770.
- Jemec GB, Heidenheim M, Nielsen NH. 1996. The prevalence of hidradenitis suppurativa and its potential precursor lesions. *J Am Acad Dermatol* 35(2 Pt 1):191-194.
- 5 Kaplan MJ. 2013. Role of neutrophils in systemic autoimmune diseases. *Arthritis Res Ther* 15(5):219.
- Kimball AB, Jemec GB, Yang M, Kageleiry A, Signorovitch JE, Okun MM, Gu Y, Wang K, Mulani P, Sundaram M. 2014. Assessing the validity, responsiveness and meaningfulness of the Hidradenitis Suppurativa Clinical Response (HiSCR) as the
10 clinical endpoint for hidradenitis suppurativa treatment. *Br J Dermatol* 171(6):1434-1442.
- Klos, A. *et al.* The role of the anaphylatoxins in health and disease. *Mol Immunol* 46, 2753-2766, doi:10.1016/j.molimm.2009.04.027 (2009).
- Kurzen H, Kurokawa I, Jemec GB, Emtestam L, Sellheyer K, Giamarellos-Bourboulis EJ, Nagy
15 I, Bechara FG, Sartorius K, Lapins J and others. 2008. What causes hidradenitis suppurativa? *Exp Dermatol* 17(5):455-456; discussion 457-472.
- Larson, R. S. & Springer, T. A. Structure and function of leukocyte integrins. *Immunol Rev* 114, 181-217 (1990).
- Lima AL, Karl I, Giner T, Poppe H, Schmidt M, Presser D, Goebeler M, Bauer B. 2016.
20 Keratinocytes and neutrophils are important sources of proinflammatory molecules in hidradenitis suppurativa. *Br J Dermatol* 174(3):514-521.
- March, D. R. *et al.* Potent cyclic antagonists of the complement C5a receptor on human polymorphonuclear leukocytes. Relationships between structures and activity. *Mol Pharmacol* 65, 868-879, doi:10.1124/mol.65.4.868 (2004).
- 25 Markiewski, M. M. *et al.* Modulation of the antitumor immune response by complement. *Nat Immunol* 9, 1225-1235, doi:10.1038/ni.1655 (2008).
- Marzano AV. 2016. Hidradenitis suppurativa, neutrophilic dermatoses and autoinflammation: what's the link? *Br J Dermatol* 174(3):482-483.
- Marzano AV, Ceccherini I, Gattorno M, Fanoni D, Caroli F, Rusmini M, Grossi A, De Simone
30 C, Borghi OM, Meroni PL and others. 2014. Association of pyoderma gangrenosum, acne, and suppurative hidradenitis (PASH) shares genetic and cytokine profiles with other autoinflammatory diseases. *Medicine (Baltimore)* 93(27):e187.
- Nemeth T, Mocsai A. 2012. The role of neutrophils in autoimmune diseases. *Immunol Lett* 143(1):9-19.
- 35 Nemeth T, Mocsai A, Lowell CA. 2016. Neutrophils in animal models of autoimmune disease. *Semin Immunol* 28(2):174-186.
- Pawaria S, Ramani K, Maers K, Liu Y, Kane LP, Levesque MC, Biswas PS. 2014. Complement component C5a permits the coexistence of pathogenic Th17 cells and type I IFN in lupus. *J Immunol* 193(7):3288-3295.
- 40 Prat L, Bouaziz JD, Wallach D, Vignon-Pennamen MD, Bagot M. 2014. Neutrophilic dermatoses as systemic diseases. *Clin Dermatol* 32(3):376-388.
- Proctor, L. M., Woodruff, T. M., Sharma, P., Shiels, I. A. & Taylor, S. M. Transdermal pharmacology of small molecule cyclic C5a antagonists. *Adv Exp Med Biol* 586, 329-345, doi:10.1007/0-387-34134-X_22 (2006).
- 45 Revuz J. 2009. Hidradenitis suppurativa. *J Eur Acad Dermatol Venereol* 23(9):985-998.
- Riedemann NC, Guo RF, Neff TA, Laudes IJ, Keller KA, Sarma VJ, Markiewski MM, Mastellos D, Strey CW, Pierson CL and others. 2002. Increased C5a receptor expression in sepsis. *J Clin Invest* 110(1):101-108.
- Riedemann, N. C. *et al.* Controlling the anaphylatoxin C5a in diseases requires a specifically
50 targeted inhibition. *Clin Immunol* 180, 25-32, doi:10.1016/j.clim.2017.03.012 (2017).

- Rittirsch D, Flierl MA, Nadeau BA, Day DE, Huber-Lang M, Mackay CR, Zetoune FS, Gerard NP, Cianflone K, Kohl J and others. 2008. Functional roles for C5a receptors in sepsis. *Nat Med* 14(5):551-557.
- 5 Slade DE, Powell BW, Mortimer PS. 2003. Hidradenitis suppurativa: pathogenesis and management. *Br J Plast Surg* 56(5):451-461.
- Smith, C. W., Marlin, S. D., Rothlein, R., Toman, C. & Anderson, D. C. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. *J Clin Invest* 83, 2008-2017, doi:10.1172/JCI114111 (1989).
- 10 Strainic MG, Shevach EM, An F, Lin F, Medof ME. 2013. Absence of signaling into CD4(+) cells via C3aR and C5aR enables autoinductive TGF-beta1 signaling and induction of Foxp3(+) regulatory T cells. *Nat Immunol* 14(2):162-171.
- Tzanetakou V, Kanni T, Giatrakou S, Katoulis A, Papadavid E, Netea MG, Dinarello CA, van der Meer JW, Rigopoulos D, Giamarellos-Bourboulis EJ. 2016. Safety and Efficacy of Anakinra in Severe Hidradenitis Suppurativa: A Randomized Clinical Trial. *JAMA Dermatol* 152(1):52-59.
- 15 Ward PA. 2009. Functions of C5a receptors. *J Mol Med (Berl)* 87(4):375-378, doi:10.1007/s00109-009-0442-7 (2009).
- Wollina U, Koch A, Heinig B, Kittner T, Nowak A. 2013. Acne inversa (Hidradenitis suppurativa): A review with a focus on pathogenesis and treatment. *Indian Dermatol Online J* 4(1):2-11.
- 20 Xu R, Wang R, Han G, Wang J, Chen G, Wang L, Li X, Guo R, Shen B, Li Y. 2010. Complement C5a regulates IL-17 by affecting the crosstalk between DC and gammadelta T cells in CLP-induced sepsis. *Eur J Immunol* 40(4):1079-1088.
- 25

CLAIMS

1. Use of a compound in the manufacture of a medicament for treating a cutaneous, neutrophilic, inflammatory disease in a subject, wherein the compound is an inhibitor of C5a activity, and
- 5 wherein the cutaneous, neutrophilic, inflammatory disease is selected from the group consisting of hidradenitis suppurativa (HS); PASH (PG, acne and hidradenitis suppurativa); PAPASH (pyogenic arthritis, acne, PG and hidradenitis suppurativa), wherein the inhibitor of C5a activity has a structure according to formula (I):

