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(56) Related Art  
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## ANSWER

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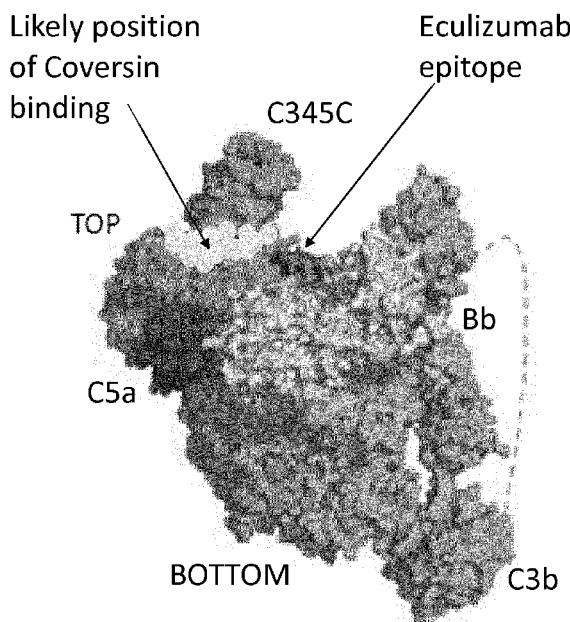
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(54) Title: ORNITHODOROS MOUBATA COMPLEMENT INHIBITOR FOR USE IN THE TREATMENT OF COMPLEMENT-MEDIATED DISEASES IN PATIENTS WITH C5 POLYMORPHISM

FIG 8



(57) Abstract: The present invention relates to methods of treating or preventing a complement-mediated disease and/or disorder in a subject with a complement C5 polymorphism, including administering to a subject in need thereof a therapeutically or prophylactically effective amount of an agent that a) inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway; and/or b) inhibits eicosanoid activity. The invention also relates to methods of identifying patient populations with C5 polymorphisms that are treatable with specific agents that a) inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway; and/or b) inhibit eicosanoid activity.



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## **ORNITHODOROS MOUBATA COMPLEMENT INHIBITOR FOR USE IN THE TREATMENT OF COMPLEMENT-MEDIATED DISEASES IN PATIENTS WITH C5 POLYMORPHISM**

### **FIELD OF THE INVENTION**

5 The present invention relates to methods of treating and preventing complement-mediated diseases and disorders in subjects with complement C5 polymorphisms.

All documents mentioned in the text and listed at the end of this description are incorporated herein by reference.

### **BACKGROUND TO THE INVENTION**

10 Reference to any prior art in the specification is not an acknowledgement or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be combined with any other piece of prior art by a skilled person in the art.

Polymorphisms are common in all but the most conserved genes in most species. The 15 presence of polymorphisms in genes that are involved in diseases and disorders of human health has led to the advent of personalised medicine. Personalised medicine allows healthcare to be customised to the individual using a variety of tools including molecular genetic analysis. Medical decisions, choice of drugs and/or treatment regimens can be tailored to the individual patient. Diagnostic testing and genotyping can be used to select 20 appropriate and optimal therapies based on the subject's individual responsiveness to particular drugs.

It has recently come to light that certain genetic variants in human C5, or C5 polymorphisms, give rise to a lack of response to certain agents that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement 25 pathway. In one clinical trial of eculizumab in a particular Japanese population of patients with the complement-mediated disorder paroxysmal nocturnal haemoglobinuria (PNH), several patients had a poor response. These patients displayed the C5 polymorphisms c.2653C>T (p.Arg885Cys) or c.2654G>A (p.Arg885His). In this type of situation, a sub-population of patients may be identified who cannot be treated by conventional means, or 30 perhaps cannot be treated at all if there is no alternative drug available, or if all known drugs act by the same mechanism.

## 1A

In the present case, there is no available alternative treatment for complement-mediated diseases and disorders that are currently treated using eculizumab. There is therefore a need to identify a means of treating the patient sub-population with C5 polymorphisms that render them currently untreatable.

5

**SUMMARY OF THE INVENTION**

Surprisingly, the present inventors have found that the tick protein Coversin (also referred to as EV576 and OmCI in the art and herein [25]) can be used to treat and prevent complement-mediated diseases and disorders in subjects with complement C5 polymorphisms.

Accordingly, the invention provides a method of treating or preventing a complement-mediated disease and/or disorder comprising administering to a subject with a complement C5 polymorphism and in need thereof a therapeutically or prophylactically effective amount of an agent that inhibits the classical complement pathway, the alternative 10 complement pathway and the lectin complement pathway.

The invention also provides an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway for treating or preventing a complement-mediated disease and/or disorder in a subject with a complement C5 polymorphism.

15 The invention also provides a method of treating or preventing a complement-mediated disease and/or disorder comprising the steps of:

- a) identifying a subject with a C5 polymorphism; and
- b) identifying an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in said subject; and
- 20 c) administering to said subject a therapeutically or prophylactically effective amount of said agent identified in step (b).

The invention also provides an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway for use in a method of treating or preventing a complement-mediated disease and/or disorder, wherein said 25 method of treating or preventing comprising the steps of:

- a) identifying a subject with a C5 polymorphism; and
- b) identifying an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in said subject; and
- 30 c) administering to said subject a therapeutically or prophylactically effective amount of said agent identified in step (b).

In a further embodiment, the invention provides a method of selecting a subject with a complement-mediated disease or disorder for treatment with a first agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in a subject with a C5 polymorphism, comprising determining the 5 effectiveness of a second agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in said subject, wherein a subject is selected for treatment with the first agent if the second agent shows decreased effectiveness in the subject with a C5 polymorphism.

In yet a further embodiment, the invention provides an agent that inhibits the classical 10 complement pathway, the alternative complement pathway and the lectin complement pathway for treating a complement-mediated disease or disorder, wherein said agent is administered to a subject on the basis the subject having been determined to have a C5 polymorphism.

### **Complement**

15 The complement system is an essential part of the body's natural defence mechanism against foreign invasion and is also involved in the inflammatory process. More than 30 proteins in serum and at the cell surface are involved in complement system function and regulation. Recently it has become apparent that, as well as the ~35 known components of the complement system which may be associated with both beneficial and pathological 20 processes, the complement system itself interacts with at least 85 biological pathways with functions as diverse as angiogenesis, platelet activation, glucose metabolism and spermatogenesis

The complement system is activated by the presence of foreign antigens. Three activation pathways exist: (1) the classical pathway which is activated by IgM and IgG complexes or 25 by recognition of carbohydrates; (2) the alternative pathway which is activated by non-self surfaces (lacking specific regulatory molecules) and by bacterial endotoxins; and (3) the lectin pathway which is activated by binding of manna-binding lectin (MBL) to mannose residues on the surface of a pathogen. The three pathways comprise parallel cascades of events that result in the production of complement activation through the formation of 30 similar C3 and C5 convertases on cell surfaces resulting in the release of acute mediators of inflammation (C3a and C5a) and formation of the membrane attack complex (MAC).

The parallel cascades involved in the classical and alternative pathways are shown in Figure 1.

The classical complement pathway, the alternative complement pathway and the lectin complement pathway are herein collectively referred to as the complement pathways.

## 5 Complement C5 polymorphisms

Several polymorphisms of human C5 have been reported [1-5]. Mutations in the gene encoding C5 have been associated with various pathologies including complement component 5 deficiency, a disease where patients show a propensity for severe recurrent infections. Defects in this gene have also been linked to susceptibility to liver fibrosis and 10 to rheumatoid arthritis. Polymorphisms in human C5 include insertions, deletions, single amino acid substitutions, frame-shifts, truncations and combinations of these changes.

Certain polymorphisms alter the interaction of C5 with inhibitors of complement pathway activation. Certain other polymorphisms alter C5 activity with clinical significance. Polymorphisms affecting Arg885 of wildtype C5 are of interest. Two polymorphisms of 15 particular interest are Arg885Cys (encoded by c.2653C>T) and p.Arg885His (encoded by c.2654G>A), both of which decrease the effectiveness of the mAb eculizumab [4].

The term “C5 polymorphism” is used herein to mean any variant of C5 other than the wild-type C5. In a human subject, the wild-type C5 is the C5 protein with accession number NP\_001726.2 ; version GI:38016947. The term “C5 polymorphism” includes insertions, 20 deletions, single or multiple amino acid substitutions, frame-shifts, truncations and combinations of these changes in the C5 protein.

These polymorphisms can be present as either heterozygous or homozygous polymorphisms, such as heterozygous C5 for a given polymorphism, homozygous for one polymorphism or heterozygous for different polymorphisms.

25 Polymorphisms of interest include changes to the amino acid sequence of wildtype C5 which are in proximity to, or within the epitope for eculizumab, (i.e. 879KSSKC883, including K879, S880, S881, K882 and/or C883). For example, any change may be in the epitope for eculizumab or up to 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 amino acids to the N- or C- terminus of the epitope for eculizumab.

Preferably, the amino acid change is not within or in proximity to the Coversin binding site of C5. This is believed to be a conserved region atop C5 $\alpha$  at the distal end of the highly conserved CUB-C5d-MG8 superdomain of C5.

Of particular interest in the present invention are C5 polymorphisms that decrease the effectiveness of one or more agents that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway in a subject with wild-type C5. By “decrease the effectiveness” it is meant that the agent has an IC<sub>50</sub> for the polymorphic C5 protein that is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 1000 or more times greater than the IC<sub>50</sub> of the same agent for the wild-type C5 protein.

10 In a preferred embodiment, the C5 polymorphism decreases the effectiveness of one or more agents that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway, but does not decrease the effectiveness of Coversin or functional equivalents thereof. In a further preferred embodiment, the C5 polymorphism decreases the effectiveness of one or more anti-C5 monoclonal antibodies

15 that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway in a subject with wild-type C5, but does not decrease the effectiveness of other agents that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway by binding to C5 without blocking the C5 convertase binding site.

20 By “does not decrease the effectiveness” it is meant that the IC<sub>50</sub> of Coversin or other agents that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway by binding to C5 without blocking the C5 convertase binding site, for the wild-type C5 protein is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of the IC<sub>50</sub> of Coversin or other agents that inhibit the classical

25 complement pathway, the alternative complement pathway and the lectin complement pathway by binding to C5 without blocking the C5 convertase binding site for the polymorphic C5 protein. The term “does not decrease” also encompasses an increase in effectiveness.

30 In an alternative embodiment, effectiveness can be measured by measuring the ability of the agent to inhibit complement activation in serum taken from the subject. For example, complement activity in the serum of said subjects can be measured by any means known in the art or described herein, for example the haemolytic assays described in reference [6].

An agent would be considered to inhibit complement activity in said subject if complement activity in the presence of the agent is reduced when compared to a control. By “reduced” in this context it is meant that complement activity in the treated sample is at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100%, reduced compared to a control.

5 In a particular embodiment, the C5 polymorphism decreases the effectiveness of monoclonal antibody agents in inhibiting activation of one or more of the complement pathways. In a particular embodiment, the C5 polymorphism decreases the effectiveness of the monoclonal antibody eculizumab in inhibiting activation of one or more of the complement pathways. In a further embodiment, the C5 polymorphism decreases the 10 effectiveness of agents that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway by blocking the C5 convertase binding site. In a further specific embodiment, the C5 polymorphism is at position Arg885. Specific polymorphisms at this position include Arg885Cys or Arg885His.

Polymorphisms that alter binding the affinity of C5 to known anti-C5 monoclonal 15 antibodies such as eculizumab, Pexelizumab, and/or N19-8, or the effectiveness of peptidic complement inhibitors such as ARC1905 are also of interest in the context of this invention.

Thus, in a specific embodiment the invention provides a method of treating or preventing a complement-mediated disease and/or disorder comprising administering to a subject with a 20 complement C5 polymorphism and in need thereof, a therapeutically or prophylactically effective amount of an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway, wherein the complement C5 polymorphism decreases the effectiveness of agents that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway by 25 blocking the C5 convertase binding site, but does not decrease the effectiveness of agents that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway without blocking the C5 convertase binding site.

Thus, in a specific embodiment the invention provides a method of treating or preventing a complement-mediated disease and/or disorder comprising administering to a subject with a 30 complement C5 polymorphism and in need thereof, a therapeutically or prophylactically effective amount of an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway, wherein the complement C5

polymorphism decreases the effectiveness of monoclonal antibodies that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway but does not decrease the effectiveness of Coversin or functional equivalents of this agent.

- 5 In this specific embodiment, the invention also provides a therapeutically or prophylactically effective amount of an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway for treating or preventing a complement-mediated disease and/or disorder in a subject with a complement C5 polymorphism, wherein the complement C5 polymorphism decreases the 10 effectiveness of agents that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway by blocking the C5 binding site, but does not decrease the effectiveness of agents that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway without blocking the C5 binding site.
- 15 In this specific embodiment, the invention also provides a therapeutically or prophylactically effective amount of an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway for treating or preventing a complement-mediated disease and/or disorder in a subject with a complement C5 polymorphism, wherein the complement C5 polymorphism decreases the 20 effectiveness of monoclonal antibodies that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway but does not decrease the effectiveness of Coversin or functional equivalents of this agent.

Thus, in a further specific embodiment the invention provides a method of treating or preventing a complement-mediated disease and/or disorder comprising administering to a 25 subject with a complement C5 polymorphism and in need thereof a therapeutically or prophylactically effective amount of an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway, wherein the complement C5 polymorphism is at position Arg885 and wherein the agent is a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence in 30 SEQ ID NO: 2 or is a functional equivalent of this protein.

In this specific embodiment, the invention also provides a therapeutically or prophylactically effective amount of an agent that inhibits the classical complement

pathway, the alternative complement pathway and the lectin complement pathway for treating or preventing a complement-mediated disease and/or disorder in a subject with a complement C5 polymorphism wherein the complement C5 polymorphism is at position Arg 885 and wherein the agent is a protein comprising or consisting of amino acids 19 to 5 168 of the amino acid sequence in SEQ ID NO: 2 or is a functional equivalent of this protein.

### **Identifying subjects for treatment**

The present invention is particularly useful in subjects that have a polymorphism in complement C5. The subject may either be already known to have a C5 polymorphism, or 10 may be suspected of having a C5 polymorphism. A subject may be suspected of having a polymorphism in C5 for example because of clinical signs of a complement-mediated disorder, because of ethnic origin or pedigree with an incidence of C5 polymorphisms, or because of unexpectedly poor response, and/or unexpectedly high response, to an agent that inhibits one of the complement pathways.

15 The invention may be useful in the sub-population of subjects that have an unexpectedly poor response to one or more agents that inhibit one of the complement pathways. In particular, the invention is useful in sub-population of subjects with a C5 polymorphism that decreases the effectiveness of monoclonal antibody agents in inhibiting activation of one or more of the complement pathways. In a particular embodiment, the C5 20 polymorphism decreases the effectiveness of the monoclonal antibody eculizumab in inhibiting activation of one or more of the complement pathways.

For example, subjects with two C5 polymorphisms at position Arg885 (c.2653C>T (p.Arg885Cys) and c.2654G>A (p.Arg885His)) do not respond to eculizumab. However, Coversin has been shown to be able to inhibit C5 cleavage and activation of the 25 complement pathways even in these subjects. Coversin interacts with complement C5 protein in a different manner to the known anti-C5 mAbs, and it is therefore expected that Coversin will also be useful in sub-populations of subjects that are not responsive to known anti-C5 mAbs, and in subjects that have other C5 polymorphisms. Coversin binds to C5, which results in stabilization of the global conformation of C5 but does not block 30 the C5 convertase cleavage site [7]. In contrast, eculizumab blocks the C5 convertase binding site [8].

The polymorphisms Arg885Cys and Arg885His are particularly prevalent in subjects of Japanese and Han Chinese origin. Coversin is therefore a particularly advantageous choice of agent in a sub-population with these ethnic origins.

As can be seen from the Examples, these polymorphisms are not limited to subjects of Japanese and Han Chinese origin. Subjects with C5 polymorphisms can also be identified by other routine techniques including molecular genetic analysis of the gene encoding the C5 protein including sequencing of the gene [4]; testing the ability of various agent to inhibit complement activation in the subject as described herein or by other methods known in the art; and/or biochemical analysis of the C5 protein from the subject, including isoelectric focusing and functional detection [9]. In a clinical setting, a subject with a C5 polymorphism may be identified by an unexpectedly poor response to an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway.

It is also anticipated that Coversin will be useful in sub-populations of subject that are unexpectedly sensitive to other agents that inhibit one of the complement pathways. For example, if a polymorphism increases the affinity of another agent, such as eculizumab, for the C5 protein, it may be difficult to dose the agent correctly. Activation of complement must be tightly controlled to prevent damage to the body's own tissues, and therefore Coversin would be a more attractive alternative in this scenario.

Once a subject with a C5 polymorphism has been identified, it is possible to identify an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in said subject. In order to identify an agent that inhibits the complement pathways, complement activity in the serum of the subject is assessed in the presence and absence of a variety of agents that inhibit the classical complement pathway, the alternative complement pathway and the lectin complement pathway, as described herein. In one specific embodiment, the agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in said subject is Coversin or a functional equivalent thereof.

Complement activity in the serum of said subjects can be measured by any means known in the art or described herein, for example the haemolytic assays described in reference [10] and/or by using the Quidel CH50 method as referred to in the examples. An agent would be considered to inhibit complement activity in said subject if complement activity

in the presence of the agent is reduced when compared to a control. By “reduced” in this context it is meant that complement activity in the treated sample is at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, reduced compared to a control.

The invention therefore provides a method of treating or preventing a complement-mediated disease and/or disorder comprising the steps of:

- a) identifying a subject with a C5 polymorphism; and
- b) identifying an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in said subject; and
- c) administering to said subject a therapeutically or prophylactically effective amount of said agent identified in step (b).

The invention also provides a therapeutically or prophylactically effective amount of an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway for use in a method of treating or preventing a complement-mediated disease and/or disorder, wherein said method of treating or preventing comprising the steps of:

- a) identifying a subject with a C5 polymorphism; and
- b) identifying an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in said subject; and
- c) administering to said subject a therapeutically or prophylactically effective amount of said agent identified in step (b).

In yet a further embodiment, the invention provides an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in a subject with a C5 polymorphism for treating a complement-mediated disease or disorder, wherein said agent is administered to a subject on the basis of the subject having been determined to have a C5 polymorphism.

In a further specific embodiment, the invention provides agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in a subject with a C5 polymorphism for treating a complement-mediated disease or disorder in a subject, wherein

- 30 a) a biological sample from said subject is assayed for the presence or absence of a C5 polymorphism, and

b) a therapeutically effective amount of said agent is selectively administered to the individual on the basis of the presence of the C5 polymorphism.

In a specific embodiment, the subject with a C5 polymorphism is identified by a lack of response to a monoclonal antibody that inhibits the classical complement pathway, the 5 alternative complement pathway and the lectin complement pathway in wild-type subjects. This sub-population of subjects is referred to as “non-responders”. Non-responders can be identified by confirming that serum complement activity is at least 60% of normal serum complement activity in the presence of the monoclonal antibody that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement 10 pathway in a subject with wild-type C5.

Of particular interest in the present invention are subjects that are non-responders to eculizumab, Pexelizumab, N19-8 and/or ARC1095.

In further specific embodiments, the specific C5 polymorphism may be identified or confirmed by sequencing the gene encoding C5 or by other molecular genetic analysis.

15 In a further embodiment, the invention provides a method of selecting a subject with a complement-mediated disease or disorder for treatment with a first agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in a subject with a C5 polymorphism, comprising determining the effectiveness in said subject of a second agent that inhibits the classical complement 20 pathway, the alternative complement pathway and the lectin complement pathway in a wild-type subject, wherein a subject is selected for treatment if the second agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in a wild-type subject shows decreased effectiveness in the subject with a C5 polymorphism.

25 Inhibition of the classical complement pathway, the alternative complement pathway and the lectin complement pathway in said subject can be measured by measuring the ability of an agent to prevent complement activation in serum from the subject, as described herein.

In a specific embodiment, the invention provides an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement 30 pathway for treating a complement-mediated disease or disorder, wherein said agent is administered to a subject on the basis of a sample from the subject having been determined to have at least 60% of normal serum complement activity in the presence of an anti-C5

monoclonal antibody that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in subjects with wild-type C5.

In a further specific embodiment, the invention provides an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement

5 pathway for treating a complement-mediated disease or disorder in a subject, wherein

a) a biological sample from said subject is assayed for the presence or absence of at least 60% of normal serum complement activity in the presence of an anti-C5 monoclonal antibody that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in subjects with wild-type C5, and

10 b) a therapeutically effective amount of said agent is selectively administered to the individual on the basis of the presence of at least 60% of normal serum complement activity in the presence of an anti-C5 monoclonal antibody that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in subjects with wild-type C5.

15 By “at least 60% of normal serum complement activity in the presence of an anti-C5 monoclonal antibody” it is meant that the serum complement activity of the subject is at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or more of the serum complement activity of a normal, untreated control subject. The control subject may have wild-type C5 or may be the same subject prior to treatment with the anti-C5 monoclonal  
20 antibody.

In some embodiments, the anti-C5 monoclonal antibody is eculizumab, Pexelizumab and/or N19-8.

These methods can be used to identify subjects and populations of subjects who are susceptible to treatment with Coversin and functional equivalents thereof.

## 25 **Complement-mediated diseases and disorders**

Activation of complement must be tightly controlled to prevent damage to the body's own tissues. Failure to control complement activation has been shown to play a role in a variety

of diseases including, amongst others, acute pancreatitis, Age Related Macular Degeneration (AMD), atypical haemolytic uremic syndrome (aHUS), Alzheimer's

30 disease, Huntingdon's disease, Parkinson's disease, allergic encephalomyelitis, allotransplantation, asthma, adult respiratory distress syndrome, influenza, burn injuries,

Crohn's disease, glomerulonephritis, haemolytic anaemia, haemodialysis, hereditary angioedema, ischaemia reperfusion injuries, multiple system organ failure, multiple sclerosis, myasthenia gravis, myocardial infarction, paroxysmal nocturnal haemoglobinuria (PNH), psoriasis, rheumatoid arthritis, septic shock, systemic lupus erythematosus, stroke, 5 thrombotic thrombocytopenic purpura (TTP), traumatic brain injury, vascular leak syndrome, and transplantation rejection and graft versus host disease (GvHD), as well as various other peripheral nerve disorders and respiratory disorders [11-16].

Peripheral nerve disorders as listed in reference 15 include of post-infective demyelinating polyradiculoneuropathy (Guillain Barré syndrome), Miller Fisher syndrome, acute 10 inflammatory demyelinating polyradiculoneuropathy (AIDP), chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), diabetic neuropathy, uraemic pruritus, multifocal motor neuropathy, paraproteinæmic neuropathy, anti-Hu neuropathy, post-diphtheria demyelinating neuropathy, multiple sclerosis, radiation myelopathy, giant cell arteritis (temporal arteritis), transverse myelitis, motor neurone disease, dermatomyositis.

15 Respiratory disorders as listed in reference 14 include asthma, including severe and steroid resistant asthma, COPD, immune complex alveolitis including those caused by exposure to organic dusts, moulds, airborne allergens, mineral dust, chemicals etc. Further conditions included in the definition of respiratory disorders include: farmer's lung, pigeon or bird fancier's lung, barn fever, miller's lung, metalworker's lung, humidifier fever, silicosis, 20 pneumoconiosis, asbestosis, byssinosis, berylliosis, mesothelioma, rhinitis, alveolitis or diffuse fibrotic lung disease caused by exposure to systemic or inhaled drugs and chemical agents including but not limited to: bleomycin, mitomycin, penicillins, sulphonamides, cephalosporins, aspirin, NSAIDs, tartrazine, ACE inhibitors, iodine containing contrast media, non-selective  $\beta$  blocking drugs, suxamethonium, hexamethonium, thiopentone, 25 amiodarone, nitrofurantoin, paraquat, oxygen, cytotoxic agents, tetracyclines, phenytoin, carbamazepine, chlorpropamide, hydralazine, procainamide, isoniazid, *p*-aminosalicylic acid. Furthermore, the term includes physical lung damage including but not limited to: crush injury, smoke and hot gas inhalation, blast injury, radiation injury, aspiration pneumonitis, lipoid pneumonia; lung damage associated with organ transplantation 30 including but not limited to: cardiac transplantation, lung transplantation, bone marrow transplantation. Also included within the definition of respiratory disorder are cryptogenic fibrosing alveolitis, allergic granulomatosis (Churg-Strauss syndrome), wegener's granulomatosis, broncholitis obliterans, interstitial pulmonary fibrosis, cystic fibrosis.

Also included are respiratory manifestations of autoimmune and connective tissue diseases including but not limited to: rheumatoid disease, systemic lupus erythematosus, systemic sclerosis, polyarteritis nodosa, polymyositis, dermatomyositis, sjögren's syndrome, ankylosing spondylitis, caplan's syndrome, goodpasture's syndrome, pulmonary alveolar 5 proteinosis, idiopathic pulmonary haemosiderosis, histiocytosis X, pulmonary infiltration with eosinophilia (PIE) including but not limited to: simple pulmonary eosinophilia, prolonged pulmonary eosinophilia, asthmatic bronchopulmonary eosinophilia, allergic bronchopulmonary aspergillosis, aspergilloma, invasive aspergillosis, tropical pulmonary eosinophilia, hypereosinophilic syndrome, parasitic infestation and 10 lymphangioleiomyomatosis (LAM).

Of particular interest in the present invention are paroxysmal nocturnal haemoglobinuria (PNH), graft versus host disease (GvHD), thrombotic thrombocytopenic purpura (TTP) and atypical haemolytic uremic syndrome (aHUS).

#### **Agent to be used in the invention**

- 15 In one aspect of the invention, the agent may bind complement C5, including complement C5 from subjects with complement C5 polymorphisms. The agent may act to prevent the cleavage of complement C5, including complement C5 from subjects with complement C5 polymorphisms, by C5 convertase into complement C5a and complement C5b-9. The agent may act to reduce C5a levels in a subject compared to an untreated subject.
- 20 In one aspect of the invention, the agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway binds to C5 in such a way as to stabilize the global conformation of C5 but not block the C5 convertase cleavage site. Binding of Coversin to C5 results in stabilization of the global conformation of C5 but does not block the convertase cleavage site.
- 25 The complement C5 protein, also referred to herein as C5, is cleaved by the C5 convertase enzyme, itself formed from C3a, an earlier product of the alternative pathway (Figure 1). The products of this cleavage include an anaphylatoxin C5a and a lytic complex C5b - 9 also known as membrane attack complex (MAC). C5a is a highly reactive peptide implicated in many pathological inflammatory processes including neutrophil and 30 eosinophil chemotaxis, neutrophil activation, increased capillary permeability and inhibition of neutrophil apoptosis [17].

MAC is associated with other important pathological processes including rheumatoid arthritis [18;19], proliferative glomerulonephritis [20], idiopathic membranous nephropathy [21], proteinuria [22], demyelination after acute axonal injury [23] and is also responsible for acute graft rejection following xenotransplantation [24].

- 5 Monoclonal antibodies and small molecules that bind and inhibit C5 have been developed to treat various diseases [12], in particular PNH, psoriasis, rheumatoid arthritis, systemic lupus erythematosus and transplant rejection. However, these monoclonal antibodies do not bind to certain C5 proteins from subjects with C5 polymorphisms, and are thus ineffective in these subjects [4].
- 10 In contrast, the Coversin, and functional equivalents thereof, inhibit complement C5 cleavage both in subjects with wild-type C5 and in subjects with C5 polymorphisms.

The ability of an agent to bind C5, including C5 from subjects with C5 polymorphisms, may be determined by standard *in vitro* assays known in the art, for example by western blotting following incubation of the protein on the gel with labelled C5. Preferably, the 15 agent according to the invention binds C5, either wild-type and/or C5 from subjects with C5 polymorphisms, with an IC<sub>50</sub> of less than 0.2 mg/ml, preferably less than 0.1 mg/ml, preferably less than 0.05 mg/ml, preferably less than 0.04 mg/ml, preferably less than 0.03 mg/ml, preferably 0.02 mg/ml, preferably less than 1 $\mu$ g/ml, preferably less than 100ng/ml, preferably less than 10ng/ml, more preferably still, less than 1ng/ml. The agent need not 20 have the same affinity for wild-type C5 and C5 from subjects with C5 polymorphisms. It may show higher, lower or the same affinity for wild-type C5 and C5 from subjects with C5 polymorphisms.

The ability of an agent to inhibit complement activation may be determined by measuring the ability of the agent to inhibit complement activation in serum. For example, 25 complement activity in the serum can be measured by any means known in the art or described herein.

According to one embodiment of the invention, the agent that binds C5 is not an anti-C5 monoclonal antibody.

The invention also provides a method of treating or preventing a complement-mediated 30 disease and/or disorder in a subject with a complement C5 polymorphism comprising

administering to a subject in need thereof a therapeutically or prophylactically effective amount of an agent that inhibits eicosanoid activity.

The invention also provides a therapeutically or prophylactically effective amount of an agent that inhibits eicosanoid activity for treating or preventing a complement-mediated  
5 disease and/or disorder in a subject with a complement C5 polymorphism.

The agent according to this aspect of the invention may inhibit leukotriene B4 (LTB4) activity. In particular, the agent according to this aspect of the invention may bind LTB4. The ability of an agent to bind LTB4 may be determined by standard *in vitro* assays known in the art, for example by western blotting following incubation of the protein on the gel  
10 with labelled LTB4. The agent according to the invention may bind LTB4 with an IC<sub>50</sub> of less than 0.2 mg/ml, preferably less than 0.1 mg/ml, preferably less than 0.05 mg/ml, preferably less than 0.04 mg/ml, preferably less than 0.03 mg/ml, preferably 0.02 mg/ml, preferably less than 1 $\mu$ g/ml, preferably less than 100ng/ml, preferably less than 10ng/ml, more preferably still, less than 1ng/ml.

15 In one aspect, the invention provides a method of treating or preventing a complement-mediated disease and/or disorder in a subject with a complement C5 polymorphism comprising administering to a subject in need thereof a therapeutically or prophylactically effective amount of an agent that:

20 a) inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway; and/or  
b) inhibits eicosanoid activity.

The invention also provides an agent that inhibits:

25 a) the classical complement pathway, the alternative complement pathway and the lectin complement pathway; and/or  
b) eicosanoid activity,

for treating or preventing a complement-mediated disease and/or disorder in a subject with a complement C5 polymorphism.

According to one embodiment of this aspect of the invention, the agent binds all of C5, C5 from subjects with C5 polymorphisms, and LTB4. The agent according to this embodiment  
30 may thus act to prevent the cleavage of complement C5 by C5 convertase into complement

C5a and complement C5b-9 (MAC), and also to inhibit LTB4 activity. Using an agent which binds to both C5 and LTB4 is particularly advantageous. C5 and the eicosanoid pathway are both believed to contribute to the observed pathology in many complement-mediated diseases and disorders. Thus by using a single agent which inhibits multiple pathways involved in the inflammatory effects of complement-mediated diseases and disorders, an enhanced effect can be achieved, compared to using an agent which inhibits only a single pathway involved in the inflammatory effects of complement-mediated diseases and disorders. There are furthermore practical advantages associated with administering a single molecule.

10 Preferably, the agent of the invention is derived from a haematophagous arthropod. The term “haematophagous arthropod” includes all arthropods that take a blood meal from a suitable host, such as insects, ticks, lice, fleas and mites. Preferably, the agent is derived from a tick, preferably from the tick *Ornithodoros moubata*.

According to one embodiment of the invention, the agent is a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or is a functional equivalent of this protein. The agent may be a protein consisting of amino acids 19 to 168 of the amino acid sequence in Figure 2 or be a functional equivalent of this protein.

According to an alternative embodiment, the protein used according to this embodiment of the invention may comprise or consist of amino acids 1 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2), or be a functional equivalent thereof. The first 18 amino acids of the protein sequence given in Figure 2 form a signal sequence which is not required for C5 binding or for LTB4 binding activity and so this may optionally be dispensed with, for example, for efficiency of recombinant protein production.

The protein having the amino acid sequence given in Figure 2, also referred to herein as the Coversin protein, was isolated from the salivary glands of the tick *Ornithodoros moubata*. Coversin is an outlying member of the lipocalin family and is the first lipocalin family member shown to inhibit complement activation. The Coversin protein inhibits the alternative, classical and lectin complement pathways by binding C5 and preventing its cleavage by C5 convertase into Complement C5a and Complement C5b – 9, thus inhibiting both the action of C5a peptide and the MAC. The Coversin protein also binds LTB4. The term “Coversin protein”, as used herein, refers to the sequence given in Figure 2 with or without the signal sequence.

The Coversin protein and the ability of this protein to inhibit complement activation has been disclosed in [25], where the Coversin protein was referred to as the "OmCI protein". The Coversin protein has also been shown to be effective in the treatment of myasthenia gravis [13], respiratory disorders [14] and peripheral nerve disorders [15]. The ability of 5 the Coversin protein to bind eicosanoids including LTB4 and its use in the treatment of diseases mediated by a leukotriene or hydroxyeicosanoid has been suggested in [26]. None of these disclosures suggest that the Coversin protein could be useful in the treatment or prevention of complement-mediated disorders in subjects with a C5 polymorphism.

It has now been found that the Coversin protein is surprisingly effective in the treatment 10 and prevention of complement-mediated disorders in subjects with a C5 polymorphism. The data presented herein demonstrate that, in a subject with an Arg885His polymorphism, inhibition of complement activity *in vitro* was resistant to eculizumab (30% or 30-80% complement inhibition at best) but completely sensitive to Coversin, with 100% inhibition at all concentrations tested.

15 Eculizumab therefore does not fully inhibit complement activity in serum from patients with an Arg885His polymorphism, and these patients received no clinical benefit from therapeutic treatment with eculizumab. These data show that complement inhibition in treatment of complement related disorders (for example PNH treatment) with eculizumab is inadequate to see a clinical benefit. On the contrary, Coversin has been shown to retain 20 normal effectiveness in reducing complement activity in serum from patients with this polymorphism and to exhibit effectiveness in the case studies (see Examples 2, 3 and 4). This suggests that the inhibition of complement as seen, for example, with Coversin, gives rise to clinical benefit, for example a clinical benefit may be observed when complement inhibition is at the level that may be achieved by Coversin.

25 According to a further embodiment of the invention, the agent may be a nucleic acid molecule encoding the Coversin protein or a functional equivalent thereof. For example, gene therapy may be employed to effect the endogenous production of the Coversin protein by the relevant cells in the subject, either *in vivo* or *ex vivo*. Another approach is the administration of "naked DNA" in which the therapeutic gene is directly injected into 30 the bloodstream or into muscle tissue.

Preferably, such a nucleic acid molecule comprises or consists of bases 55 to 507 of the nucleotide sequence in Figure 2 (SEQ ID NO: 1). This nucleotide sequence encodes the

Coversin protein in Figure 2 without the signal sequence. The first 54 bases of the nucleotide sequence in Figure 2 encode the signal sequence which is not required for complement inhibitory activity or LTB4 binding activity. Alternatively, the nucleic acid molecule may comprise or consist of bases 1 to 507 of the nucleic acid sequence in Figure 5 2, which encodes the protein with the signal sequence.

The Coversin protein has been demonstrated to bind to C5 and prevent its cleavage by C5 convertase in rat, mouse and human serum with an IC<sub>50</sub> of approximately 0.02mg/ml. Preferably, functional equivalents of the Coversin protein which retain the ability to bind C5 with an IC<sub>50</sub> of less than 0.2 mg/ml, preferably less than 0.1 mg/ml, preferably less than 10 0.05 mg/ml, preferably less than 0.02 mg/ml, preferably less than 1 $\mu$ g/ml, preferably less than than 100ng/ml, preferably less than 10ng/ml, more preferably still, less than 1ng/ml.

The Coversin protein has also been demonstrated to bind LTB4. Functional equivalents of the Coversin protein may also retain the ability to bind LTB4 with a similar affinity as the Coversin protein.

15 In one respect, the term “functional equivalent” is used herein to describe homologues and fragments of the Coversin protein which: a) retain its ability to bind C5, either wild-type C5 or C5 from a subject with a C5 polymorphism, and to prevent the cleavage of complement C5 by C5 convertase into complement C5a and complement C5b-9; and/or b) retain its ability to bind LTB4.

20 The term “functional equivalent” also refers to molecules that are structurally similar to the Coversin protein or that contain similar or identical tertiary structure, particularly in the environment of the active site or active sites of the Coversin protein that binds to C5, either wild-type C5 or C5 from a subject with a C5 polymorphism, and/or LTB4, such as synthetic molecules. Amino acids in Coversin that are likely to be required for LTB4 25 binding are described in [26].

The term “homologue” is meant to include reference to paralogues and orthologues of the Coversin sequence that is explicitly identified in Figure 2, including, for example, the Coversin protein sequence from other tick species, including *Rhipicephalus appendiculatus*, *R. sanguineus*, *R. bursa*, *A. americanum*, *A. cajennense*, *A. hebraicum*, *Boophilus microplus*, *B. annulatus*, *B. decoloratus*, *Dermacentor reticulatus*, *D. andersoni*, *D. marginatus*, *D. variabilis*, *Haemaphysalis inermis*, *Ha. leachii*, *Ha. punctata*, *Hyalomma anatolicum anatolicum*, *Hy. dromedarii*, *Hy. marginatum marginatum*, *Ixodes ricinus*, *I.*

*persulcatus*, *I. scapularis*, *I. hexagonus*, *Argas persicus*, *A. reflexus*, *Ornithodoros erraticus*, *O. moubata moubata*, *O. m. porcinus*, and *O. savignyi*. The term “homologue” is also meant to include the equivalent Coversin protein sequence from mosquito species, including those of the *Culex*, *Anopheles* and *Aedes* genera, particularly *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles gambiae*; flea species, such as *Ctenocephalides felis* (the cat flea); horseflies; sandflies; blackflies; tsetse flies; lice; mites; leeches; and flatworms. The native Coversin protein is thought to exist in *O. moubata* in another three forms of around 18kDa and the term “homologue” is meant to include these alternative forms of Coversin.

10 Methods for the identification of homologues of the Coversin sequence given in Figure 2 will be clear to those of skill in the art. For example, homologues may be identified by homology searching of sequence databases, both public and private. Conveniently, publicly available databases may be used, although private or commercially-available databases will be equally useful, particularly if they contain data not represented in the

15 public databases. Primary databases are the sites of primary nucleotide or amino acid sequence data deposit and may be publicly or commercially available. Examples of publicly-available primary databases include the GenBank database (<http://www.ncbi.nlm.nih.gov/>), the EMBL database (<http://www.ebi.ac.uk/>), the DDBJ database (<http://www.ddbj.nig.ac.jp/>), the SWISS-PROT protein database (<http://expasy.hcuge.ch/>), PIR (<http://pir.georgetown.edu/>), TrEMBL (<http://www.ebi.ac.uk/>), the TIGR databases (see <http://www.tigr.org/tdb/index.html>), the NRL-3D database (<http://www.nbrfa.georgetown.edu>), the Protein Data Base (<http://www.rcsb.org/pdb>), the NRDB database (<ftp://ncbi.nlm.nih.gov/pub/nrdb/README>),

20 the OWL database (<http://www.biochem.ucl.ac.uk/bsm/dbbrowser/OWL/>) and the secondary databases PROSITE (<http://expasy.hcuge.ch/sprot/prosite.html>), PRINTS (<http://iupab.leeds.ac.uk/bmb5dp/prints.html>), Profiles ([http://ulrec3.unil.ch/software/PFSCAN\\_form.html](http://ulrec3.unil.ch/software/PFSCAN_form.html)), Pfam (<http://www.sanger.ac.uk/software/pfam>), Identify (<http://dna.stanford.edu/identify/>)

25 and Blocks (<http://www.blocks.fhcrc.org>) databases. Examples of commercially-available databases or private databases include PathoGenome (Genome Therapeutics Inc.) and PathoSeq (previously of Incyte Pharmaceuticals Inc.).

Typically, greater than 30% identity between two polypeptides (preferably, over a specified region such as the active site) is considered to be an indication of functional equivalence and thus an indication that two proteins are homologous. Preferably, proteins that are homologues have a degree of sequence identity with the Coversin protein sequence 5 identified in Figure 2 (SEQ ID NO:2) of greater than 60%. More preferred homologues have degrees of identity of greater than 70%, 80%, 90%, 95%, 98% or 99%, respectively with the Coversin protein sequence given in Figure 2 (SEQ ID NO:2). Percentage identity, as referred to herein, is as determined using BLAST version 2.1.3 using the default parameters specified by the NCBI (the National Center for Biotechnology Information; 10 <http://www.ncbi.nlm.nih.gov/>) [Blosum 62 matrix; gap open penalty=11 and gap extension penalty=1].

Functional equivalents of the Coversin protein sequence given in Figure 2 include mutants containing amino acid substitutions, insertions or deletions from the wild type sequence, for example, of 1, 2, 3, 4, 5, 7, 10 or more amino acids, provided that such mutants retain the 15 ability to bind wild-type C5 and/or C5 from subjects with a C5 polymorphism. Mutants thus include proteins containing conservative amino acid substitutions that do not affect the function or activity of the protein in an adverse manner. This term is also intended to include natural biological variants (e.g. allelic variants or geographical variations within the species from which the Coversin proteins are derived). Mutants with improved ability to bind wild- 20 type C5 and/or C5 from subjects with a C5 polymorphism and/or LTB4 may also be designed through the systematic or directed mutation of specific residues in the protein sequence.

Fragments of the Coversin protein and of homologues of the Coversin protein are also embraced by the term “functional equivalents” providing that such fragments retain the ability to bind wild-type C5 and/or C5 from subjects with a C5 polymorphism and/or LTB4. 25 Fragments may include, for example, polypeptides derived from the Coversin protein sequence which are less than 150 amino acids, less than 125 amino acids, less than 100 amino acids, less than 75 amino acids, less than 50 amino acids, or even 25 amino acids or less, provided that these fragments retain the ability to bind to complement wild-type C5 and/or C5 from subjects with a C5 polymorphism and/or LTB4. Fragments may include, for example, 30 polypeptides derived from the Coversin protein sequence which are at least 150 amino acids, at least 125 amino acids, at least 100 amino acids, at least 75 amino acids, at least 50 amino acids, or at least 25 amino acids, provided that these fragments retain the ability to bind to complement wild-type C5 and/or C5 from subjects with a C5 polymorphism and/or LTB4.

Any functional equivalent or fragment thereof preferably retains the pattern of cysteine residues that is found in Coversin. For example said functional equivalent comprises six cysteine residues that are spaced relative to each other at a distance of 32 amino acids apart, 62 amino acids apart, 28 amino acids apart, 1 amino acid apart and 21 amino acids apart as arranged from the amino terminus to the carboxyl terminus of the sequence according to amino acids 1 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO:2). Exemplary fragments of Coversin protein are disclosed in SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14. The DNA encoding the corresponding fragments are disclosed in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13.

Included as such fragments are not only fragments of the *O. moubata* Coversin protein that is explicitly identified herein in Figure 2, but also fragments of homologues of this protein, as described above. Such fragments of homologues will typically possess greater than 60% identity with fragments of the Coversin protein sequence in Figure 2, although more preferred fragments of homologues will display degrees of identity of greater than 70%, 80%, 90%, 95%, 98% or 99%, respectively with fragments of the Coversin protein sequence in Figure 2. Fragments with improved may, of course, be rationally designed by the systematic mutation or fragmentation of the wild type sequence followed by appropriate activity assays. Fragments may exhibit similar or greater affinity for C5, either the wild-type or polymorphic variant of C5 or both, and/or LTB4 as Coversin.

A functional equivalent used according to the invention may be a fusion protein, obtained, for example, by cloning a polynucleotide encoding the Coversin protein in frame to the coding sequences for a heterologous protein sequence. The term “heterologous”, when used herein, is intended to designate any polypeptide other than the Coversin protein or its functional equivalent. Example of heterologous sequences, that can be comprised in the soluble fusion proteins either at N- or at C-terminus, are the following: extracellular domains of membrane-bound protein, immunoglobulin constant regions (Fc region), multimerization domains, domains of extracellular proteins, signal sequences, export sequences, or sequences allowing purification by affinity chromatography. Many of these heterologous sequences are commercially available in expression plasmids since these sequences are commonly included in the fusion proteins in order to provide additional properties without significantly impairing the specific biological activity of the protein fused to them [27]. Examples of such additional properties are a longer lasting half-life in

body fluids, the extracellular localization, or an easier purification procedure as allowed by a tag such as a histidine, GST, FLAG, avidin or HA tag.

The Coversin protein and functional equivalents thereof, may be prepared in recombinant form by expression in a host cell. Such expression methods are well known to those of skill 5 in the art and are described in detail by [28] and [29]. Recombinant forms of the Coversin protein and functional equivalents thereof are preferably unglycosylated.

The proteins and fragments of the present invention can also be prepared using conventional techniques of protein chemistry. For example, protein fragments may be prepared by chemical synthesis. Methods for the generation of fusion proteins are standard 10 in the art and will be known to the skilled reader. For example, most general molecular biology, microbiology recombinant DNA technology and immunological techniques can be found in [28] or [30].

### **Modes of administration**

Coversin and its functional equivalents do not require a medical professional for 15 administration to be carried out, and these molecules are rapidly absorbed. Many recombinant antibodies are absorbed very slowly and as a result need to be infused over long periods (e.g. intravenously). The administration of such molecules thus also requires a medical professional. Thus, as well as having the advantage of being more effective at inhibiting the activation of the complement pathways in subjects with a C5 polymorphism, 20 Coversin also possesses the advantage of being easier to administer than other agents such as antibodies like eculizumab.

The subject to which the agent is administered in the practice of the invention is preferably a mammal, preferably a human. The subject may be an adult, a child, or an infant. The subject to which the agent is administered may also be suffering from a complement- 25 mediated disease or disorder. In particular, the subject may be known to have, or be suspected of having, a complement C5 polymorphism.

The agent is administered in a therapeutically or prophylactically effective amount. The term "therapeutically effective amount" refers to the amount of agent needed to treat the complement-mediated disease or disorder, as defined elsewhere herein. The term 30 "prophylactically effective amount" used herein refers to the amount of agent needed to prevent complement-mediated disease or disorder as defined elsewhere herein. Preferably, the dose of the agent is sufficient to bind as much available C5 as possible in the subject,

more preferably, all available C5. The dose of the agent may alternatively be sufficient to bind as much available LTB4 as possible in the subject, more preferably, all available LTB4. In some aspects, the dose of the agent is sufficient to binds as much available C5 and LTB4 as possible, for example all available C5 and LTB4. The dose of the agent supplied is at least twice the molar dose needed to bind all available C5 and/or LTB4 in the subject. The dose of the agent supplied may be 2.5 times, 3 times or 4 times the molar dose needed to bind all available C5 and/or LTB4 in the subject. Preferably, the dose is from 0.0001 mg/kg (mass of drug compared to mass of patient) to 20 mg/kg, preferably 0.001 mg/kg to 10 mg/kg, preferably 0.01 mg/kg to 2 mg/kg, preferably 0.1mg/kg to 1mg/kg; alternatively 0.2mg/kg to 0.8mg/kg; alternatively 0.3mg/kg to 0.7mg/kg; alternatively 0.4mg/kg to 0.6mg/kg; for example 0.14mg/kg or 0.57mg/kg. The therapeutically or prophylactically effective amount can additionally be defined in terms of the inhibition of terminal complement, for example, an amount that means that terminal complement activity is reduced by at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 91, 92 ,93, 94, 95, 96, 97, 98, 99, 100%, compared to terminal complement activity in the absence of treatment. Dose and frequency may be adjusted in order to maintain terminal complement activity at the desired level, which may be, for example 10% or less, for example 9, 8, 7, 6, 5, 4, 3, 2, 1% or less compared to terminal complement activity in the absence of treatment.

The frequency with which the dose needs to be administered will depend on the half-life of the agent involved. Where the agent is the Coversin protein or a functional equivalent thereof, the dose may be administered as a continuous infusion, in bolus doses or on a daily basis, twice daily basis, or every two, three, four days, five, six, seven, 10, 15 or 20 days or more. As noted elsewhere, a particular advantage of the Coversin protein and its functional equivalents is the relative ease and rapidity with which it can be administered, and the fact that medical professionals are not required for administration.

Single or multiple doses may be administered. For example at least 2, 3, 4, 5, 6, 7, or 8 doses may be administered. Single doses are one embodiment. The exact dosage and the frequency of doses may also be dependent on the patient's status at the time of administration. Factors that may be taken into consideration when determining dosage include the need for treatment or prophylaxis, the severity of the disease state in the patient, the general health of the patient, the age, weight, gender, diet, time and frequency of administration, drug combinations, reaction sensitivities and the patient's tolerance or

response to therapy. The precise amount can be determined by routine experimentation, but may ultimately lie with the judgement of the clinician.

The dosage regimen may also take the form of an initial “loading dose” followed by one or more subsequent “maintenance doses”. In general, the loading dose will be greater than

5 the maintenance dose. The loading dose may be 2, 5, 10 or more times greater than the maintenance dose. The loading dose may be administered as a single dose, or as one or more doses in a particular time frame. Typically, the loading dose will be 1, 2, 3, 4 or 5 doses administered in a single 24 hour period. The maintenance dose will typically be a lower dose that is repeated at regular intervals, such as every 3, 4, 6, 8, 12, 24, or 48 hours.

10 The precise regimen can be determined by routine experimentation, but may ultimately lie with the judgement of the clinician.

The loading dose may be 0.0001mg/kg (mass of drug compared to mass of patient) to 20mg/kg, and the maintenance dose may be between 0.0001 mg/kg to 20mg/kg; alternatively the loading dose is 0.001 mg/kg to 10 mg/kg and the maintenance dose is

15 0.001 mg/kg to 10 mg/kg, alternatively the loading dose is 0.01 mg/kg to 2 mg/kg and the maintenance dose is 0.01mg/kg to 2mg/kg; alternatively the loading dose is 0.1mg/kg to 1mg/kg and the maintenance dose is 0.1mg/kg to 1mg/kg; alternatively the loading dose is 0.1mg/kg to 1mg/kg and the maintenance dose is 0.05mg/kg to 0.5mg/kg; alternatively the loading dose is 0.2mg/kg to 0.8mg/kg and the maintenance dose is 0.1mg/kg to 0.4mg/kg;

20 alternatively the loading dose is 0.3mg/kg to 0.7mg/kg and the maintenance dose is 0.1mg/kg to 0.3mg/kg; alternatively the loading dose is 0.4mg/kg to 0.6mg/kg and the maintenance dose is 0.1mg/kg to 0.2mg/kg for example where the loading dose is 0.57mg/kg and the maintenance dose is 0.14mg/kg.

The loading dose may be 0.0001mg/kg (mass of drug compared to mass of patient) to

25 20mg/kg, and the maintenance dose may be between 0.0001 mg/kg to 20mg/kg; alternatively the maintenance dose may be 0.001 mg/kg to 10 mg/kg, alternatively the maintenance dose may be 0.01mg/kg to 2mg/kg; alternatively the maintenance dose may be 0.1mg/kg to 1mg/kg; alternatively the maintenance dose may be 0.1mg/kg to 0.8mg/kg; alternatively the maintenance dose may be 0.1mg/kg to 0.6mg/kg; alternatively the

30 maintenance dose may be 0.1mg/kg to 0.4mg/kg; alternatively the maintenance dose may be 0.1mg/kg to 0.2mg/kg.

The loading dose may be 0.0001mg/kg (mass of drug compared to mass of patient) to 20mg/kg, and the maintenance dose may be between 0.0001 mg/kg to 20mg/kg; alternatively the loading dose may be 0.001 mg/kg to 10 mg/kg, alternatively the loading dose may be 0.01 mg/kg to 2 mg/kg; alternatively the loading dose may be 0.1mg/kg to 5 1mg/kg; alternatively the loading dose may be 0.1mg/kg to 1mg/kg; alternatively the loading dose may be 0.2mg/kg to 0.8mg/kg; alternatively the loading dose may be 0.3mg/kg to 0.6mg/kg; alternatively the loading dose may be 0.4mg/kg to 0.6mg/kg. The agent will generally be administered in conjunction with a pharmaceutically acceptable carrier. The term “pharmaceutically acceptable carrier”, as used herein, includes genes, 10 polypeptides, antibodies, liposomes, polysaccharides, polylactic acids, polyglycolic acids and inactive virus particles or indeed any other agent provided that the carrier does not itself induce toxicity effects or cause the production of antibodies that are harmful to the individual receiving the pharmaceutical composition. Pharmaceutically acceptable carriers may additionally contain liquids such as water, saline, glycerol, ethanol or auxiliary 15 substances such as wetting or emulsifying agents, pH buffering substances and the like. The pharmaceutical carrier employed will thus vary depending on the route of administration. Carriers may enable the pharmaceutical compositions to be formulated into tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions to aid intake by the patient. A thorough discussion of pharmaceutically acceptable carriers is available in 20 [31].

The agent may be delivered by any known route of administration. The agent may be delivered locally or systemically. The agent may be delivered by a parenteral route (e.g. by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue). The compositions can also be administered 25 into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications, needles, and hyposprays.

Preferably the agent is delivered via subcutaneous injection. In some embodiments this is via once daily subcutaneous injection, for example at an initial loading dose of between 0.0001mg/kg (mass of drug compared to mass of patient) to 20mg/kg, followed by once 30 daily maintenance doses of between 0.0001mg/kg to 20mg/kg, or other doses disclosed elsewhere herein. Alternatively the agent may be delivered via subcutaneous injection every other day.

In a preferred embodiment the agent is delivered via once daily subcutaneous injection at an initial loading dose of 0.4mg/kg-0.6mg/kg (for example 0.57mg/kg) followed by once daily maintenance doses of 0.1mg/kg-0.2mg/kg (for example 0.14mg/kg).

The agent may be administered alone or as part of a treatment regimen also involving the  
5 administration of other drugs currently used in the treatment of patients with a complement-mediated disease or disorder.

The agent may be administered simultaneously, sequentially or separately with the other drug(s). For example, the agent may be administered before or after administration of the other drug(s). In particular, the agent may be administered after a previous drug has failed to  
10 threat the complement mediated disease or disorder. In a specific embodiment, the agent may be administered after an anti-C5 monoclonal antibody.

In particular embodiments:

- (i) the complement-mediated disease is paroxysmal nocturnal haemoglobinuria (PNH);
- (ii) the complement C5 polymorphism is at residue Arg885;
- 15 (iii) the agent for treatment is Coversin protein or fragments or homologues of the Coversin protein providing that such fragments retain the ability to bind wild-type C5 and/or C5 from subjects with a C5 polymorphism;
- (iv) the agent is delivered subcutaneously.

In certain embodiments the subcutaneous injection is once daily at an initial loading dose  
20 of 0.4mg/kg-0.6mg/kg (mass of drug compared to mass of patient), followed by once daily maintenance doses of 0.1mg/kg-0.2mg/kg; more preferably at an initial loading dose of 0.57mg/kg (mass of drug compared to mass of patient), followed by once daily maintenance doses of 0.14mg/kg.

In particular embodiments:

- 25 (i) the complement-mediated disease is graft versus host disease (GvHD);
- (ii) the complement C5 polymorphism is at residue Arg885;
- (iii) the agent for treatment is Coversin protein or fragments or homologues of the Coversin protein providing that such fragments retain the ability to bind wild-type C5 and/or C5 from subjects with a C5 polymorphism;

(iv) the agent is delivered subcutaneously.

In certain embodiments the subcutaneous injection is once daily at an initial loading dose of 0.4mg/kg–0.6mg/kg (mass of drug compared to mass of patient), followed by once daily maintenance doses of 0.1mg/kg–0.2mg/kg; more preferably at an initial loading dose of

5 0.57mg/kg (mass of drug compared to mass of patient), followed by once daily maintenance doses of 0.14mg/kg.

In particular embodiments:

(i) the complement-mediated disease is thrombotic thrombocytopenic purpura (TTP);

(ii) the complement C5 polymorphism is at residue Arg885;

10 (iii) the agent for treatment is Coversin protein or fragments or homologues of the Coversin protein providing that such fragments retain the ability to bind wild-type C5 and/or C5 from subjects with a C5 polymorphism;

(iv) the agent is delivered subcutaneously.

In certain embodiments the subcutaneous injection is once daily at an initial loading dose

15 of 0.4mg/kg–0.6mg/kg (mass of drug compared to mass of patient), followed by once daily maintenance doses of 0.1mg/kg–0.2mg/kg; more preferably at an initial loading dose of 0.57mg/kg (mass of drug compared to mass of patient), followed by once daily maintenance doses of 0.14mg/kg.

In particular embodiments:

20 (i) the complement-mediated disease is atypical haemolytic uremic syndrome (aHUS);

(ii) the complement C5 polymorphism is at residue Arg885;

(iii) the agent for treatment is Coversin protein or fragments or homologues of the Coversin protein providing that such fragments retain the ability to bind wild-type C5 and/or C5 from subjects with a C5 polymorphism;

25 (iv) the agent is delivered subcutaneously.

In certain embodiments the subcutaneous injection is once daily at an initial loading dose of 0.4mg/kg–0.6mg/kg (mass of drug compared to mass of patient), followed by once daily maintenance doses of 0.1mg/kg–0.2mg/kg; more preferably at an initial loading dose of

0.57mg/kg (mass of drug compared to mass of patient), followed by once daily maintenance doses of 0.14mg/kg.

Various aspects and embodiments of the present invention will now be described in more detail by way of example. It will be appreciated that modification of detail may be made  
5 without departing from the scope of the invention.

**BRIEF DESCRIPTION OF FIGURES:**

**Figure 1:** Schematic diagram of classical and alternative pathways of complement activation. Enzymatic components, dark grey. Anaphylatoxins enclosed in starbursts.

**Figure 2:** Primary sequence of Coversin. Signal sequence underlined. Cysteine residues in 5 bold type. Nucleotide and amino acid number indicated at right.

**Figure 3:** Peak and trough platelet counts from patient treated in Example 2

**Figure 4:** *In vitro* testing of serum from patient in Example 4 by CH50 assay after spiking with variable doses of Coversin and eculizumab (expressed as a percentage of control). Shows percentage complement activity in serum from patient in Example 4 compared to 10 control serum in presence of Eculizumab or Coversin. KEY: Ecu, spiked with Eculizumab; Cov, spiked with Coversin. NC3, normal control serum; R2, patient serum.

**Figure 5:** *In vitro* testing of serum from patient in Example 4 by CH50 assay after spiking with variable doses of Coversin and eculizumab. Shows complement activity in CH50 Eq/ml units in serum from patient in Example 4 compared to control serum in presence of 15 Eculizumab or Coversin. KEY: Ecu, spiked with Eculizumab; Cov, spiked with Coversin. NC3, normal control serum; R2, patient serum.

**Figure 6:** *In vitro* testing of serum from patient in Example 3 by CH50 assay after spiking with variable doses of Coversin and eculizumab (expressed as a percentage of control) Shows percentage complement activity from patient in Example 3 compared to control 20 serum in presence of Eculizumab or Coversin. KEY: Ecu, spiked with Eculizumab; Cov, spiked with Coversin. NC, normal control serum; BJ1, replicate 1 using patient serum; BJ2 replicate 2 using patient serum.

**Figure 7:** *In vitro* testing of serum from patient in Example 3 by CH50 assay after spiking with variable doses of Coversin and eculizumab. Shows complement activity in CH50 25 Eq/ml units from patient in Example 3 in presence of Eculizumab or Coversin. KEY: Ecu, spiked with Eculizumab; Cov, spiked with Coversin. BJ1 and BJ2 referred to as Pat 1a and Pat 1b.

**Figure 8:** Molecular model showing the position of the eculizumab epitope and the likely binding site of Coversin

## EXAMPLES

### Example 1 – *in vitro* inhibition of C5 activity

Terminal complement activity was measured in serum from a 4 year old, male, Caucasian  
5 patient found to have a rare genetic polymorphism in the gene encoding complement C5  
(c.2654G>A (p.Arg885His)) by Quidel CH<sub>50</sub> haemolysis assay.

The Quidel Microvue CH50 Eq enzyme immunoassay (cat #A018) was used is for *in vitro*  
measurement of total classical pathway activity in human serum.  
[http://www.quidel.com/sites/quidel.com/files/product/documents/a018\\_microvue\\_ch50\\_eq\\_english\\_1.pdf](http://www.quidel.com/sites/quidel.com/files/product/documents/a018_microvue_ch50_eq_english_1.pdf)

The kit provides a direct measure of the terminal complement complex (TCC) formation  
under standard conditions. Measurement of CH50 with the kit has 3 steps:

1. Activation of the classical complement pathway in undiluted serum resulting in  
15 formation of TCC.
2. Dilution of serum and addition to microassay wells coated with an antibody that  
captures TCC.
3. Quantification of captured TCC with and anti-TCC horse radish peroxidase (HRP)  
conjugated antibody.

20

Colour intensity on addition of substrate is proportional to the concentration of TCC  
present in each reaction. Using the kit standard curve (determined during each assay)  
assay results are expressed in CH50 unit equivalents per millilitre (CH50 U Eq/ml).

25 The linear range for the kit is 30-310 U Eq/ml.

According to the manufacturers the cut off for normality determined from 234 individual  
human samples is 70 CH50 U Eq/ml.

Following treatments with eculizumab, the patient retained 70% complement activity as compared to complement activity in the serum of a normal control with wild-type C5.

Spiking the serum taken after administration of eculizumab with 30, 60 and 120 $\mu$ g/ml Coversin resulted in undetectable levels of complement activity.

- 5 Thus, in a non-responder to eculizumab, Coversin retained normal effectiveness.

### **Example 2 – case study**

A 4 year old, male, Caucasian patient, weighing 13.6 kg, received a primary diagnosis of chronic granulomatous disease and underwent haematopoietic stem cell transplantation in October 2013. Subsequently the patient developed major gastrointestinal bleeding due to 10 thrombocytopenia and is now receiving daily platelet transfusions. The diagnosis is either graft versus host disease (GvHD) or thrombotic thrombocytopenic purpura (TTP).

Treatment with eculizumab, infliximab and rituximab have been unsuccessful.

The patient has been found to have a rare genetic polymorphism in the gene encoding complement C5 (c.2654G>A (p.Arg885His)), previously only described in people of 15 Japanese or Han Chinese origin.

*In vitro* assays of serum complement activity as described above showed that complement activity result was ~70% haemolytic activity compared to normal control after treatment with eculizumab. In contrast, spiking the serum with Coversin at 30, 60 and 120 $\mu$ g/ml reduced haemolytic activity to undetectable levels.

- 20 Following identification of susceptibility to inhibition of the complement pathways by Coversin, the following treatment was begun:

Coversin, by subcutaneous injection according to the following schedule:

Initial Loading Dose: 0.57mg/kg = 7.8mg (0.7ml)

Maintenance Dose: 0.14mg/kg = 1.9mg (0.2ml) every 24 hours thereafter

- 25 Serum will be taken daily for complement activity and dose and/or frequency will be adjusted in order to maintain terminal complement activity at 10% or less compared to normal control serum.

The following outcomes will also be monitored:

- a) Change in trough platelet counts

- b) Change in serum LDH
- c) Terminal complement activity measured by Quidel CH<sub>50</sub> haemolysis assay

**Example 3 – results of case study**

The patient of Example 2 was treated with Coversin for about 6 weeks. On the first day of

5 treatment he received a dose calculated to ablate circulating C5 (0.57mg/kg) and thereafter 50% of this dose until the end of the second week. From then the patient received the same dose every other day for two weeks and then half that dose for a further two weeks. It should be noted that the dose from the third week onwards was likely to have been inadequate to fully control terminal complement activity.

10 Clinically the patient stabilised during the period that he received the full dose. The main consequence of his illness, which was presumed to be a thrombotic thrombocytopenic purpura (TTP), was severely reduced platelet count for which he had been receiving two units of platelets every day for several months. After 7 days of Coversin treatment the

15 trough platelet counts (approximately 12 hours post platelet transfusion) began to rise reaching 98,000 by Day 14, the highest value that had been recorded throughout his illness.

His requirement for platelet transfusion was reduced to one unit per day at that point (see Figure 3).

The dose was reduced at the start of the third week and the trough platelet count fell to below 50,000 and did not recover for the remainder of his illness. The rise in trough

20 platelet count and the reduced need for platelet transfusion was considered by the medical staff as a clear indication of a positive response to Coversin. The deterioration after the dose was reduced seems to confirm this.

The final dose of Coversin was given after 6 weeks and the patient rapidly deteriorated and died from perforation of the jejunum after a further 2 weeks.

25

**Example 4 – case study**

A male patient aged in his mid-forties was diagnosed with PNH and he has been treated with eculizumab for about a year with an inadequate clinical response. Genetic analysis has confirmed a heterozygous C5 polymorphism at position c.2654 but it is not known what

30 amino acid shift this gives rise to although it is known that it is not pArg885His.

**Example 5 - Terminal complement activity in serum from patients***Reagents & Samples*

Sample Preparation: Serum was prepared by collecting blood into plain glass or SST

5 Vacutainer tubes (or equivalent) and allowing it to clot for 1 hour, before centrifugation at 1500g for 10 minutes. The serum was separated immediately (avoiding contamination with any blood cells) and stored in screw cap cryotubes (approximately 0.5ml aliquots) at -70°C.

Coversin: Frozen 10.9mg/ml solution at -70°C. Dilute 10uL in 90uL normal control or

10 patient serum to give a final concentration of 1.09mg/ml. Dilute 10uL in 90uL autologous serum to give a final concentration of 109ug/ml. Double dilute in autologous serum to achieve a final concentration range of: 0.4 - 54.5ug/ml.

Eculizumab: Frozen solution of 10mg/ml. Dilute 10uL in 90uL normal control or patient

15 serum to give a final concentration of 1mg/ml. Dilute 10uL in 90uL autologous serum to give a final concentration of 100ug/ml. Double dilute in autologous serum to achieve a final concentration range of: 0.4 - 50ug/ml.

Buffer: Phosphate Buffered Saline (0.01M phosphate buffer, 0.0027M potassium chloride,

20 0.137M sodium chloride, pH7.4).

*Methods*

Coversin, Eculizumab, or buffer (control), are spiked into serum according to the

procedure above to achieve a range of final concentrations. These are then assayed for

25 CH50 Equivalent activity using the Quidel CH50 kit, using duplicate wells.

*Results*

Calculate CH50 values from the calibration curve provided with the kit. Plot the results as raw CH50 values against C5 inhibitor concentration.

Calculate the CH50 result at each C5 inhibitor concentration as a percentage of the CH50 concentration of the relevant buffer control. Plot the percentage CH50 results against inhibitor concentration.

5

Repeat the experiment on separate days to obtain 3 measurements in each patient and in a single normal control. This provides an estimate of between experiment variability.

Repeat the experiment on separate days in single experiments on 6 different normal  
10 controls. This provides an estimate of between subject responsiveness (and avoids the risk of using a single subject who may have an unknown C5 mutation or polymorphism).

The highest dose of each drug to whole serum was added and then two-fold serial dilutions were made in whole serum. One replicate was used for each drug dose.

The highest dose of Eculizumab was 50 $\mu$ g/ml, then 25, 12.5, 6.3, 3.2, 1.6, 0.8, 0.4 and  
15 0 $\mu$ g/ml. The highest dose of Coversin was 54.5 $\mu$ g/ml, then 27.3, 13.1, 6.6, 3.3, 1.7, 0.9 and 0 $\mu$ g/ml.

After serial dilution the serum was activated and assayed in accordance with the instructions for the Quidel CH50 kit.

CH50 U Eq/ml were calculated in comparison with the kit standards and plotted against  
20 drug concentration for each of the three serum samples and two drug treatments. They were also plotted as a percentage of the CH50 value of the relevant buffer only control.

Normal human serum and serum from the patients in the case studies were tested for terminal complement activity in the presence of Eculizumab and Coversin as described  
25 above.

As shown in Figures 6 and 7, in the absence of either drug the baseline CH50 values of the normal human serum (average 78.1 CH50 U Eq/ml) and the two patient serum samples from the patient described in the case study of examples 2 and 3 (average 82.4 and 60.6

CH50 U Eq/ml) were within (normal control and BJ 2) or slightly below (BJ 1) the normal human range of >70 CH50 U Eq/ml.

5 Coversin inhibited both normal human serum and serum from the patient with the p.Arg885His polymorphism equally well. Less than 5% of baseline CH50 (U Eq/ml) was seen at Coversin concentrations of approximately 15ug/ml.

10 Eculizumab inhibited normal human serum at the expected dose, with less than 5% of baseline CH50 (U Eq/ml) seen at concentrations of approximately 45ug/ml. At doses above 25ug/ml eculizumab inhibited complement activity measured using the Quidel CH50 kit similarly in normal human serum and serum from the patient with the p.Arg885His polymorphism. However, it did not fully inhibit serum from the patient, with approximately 20% of baseline CH50 remaining at the highest dose of eculizumab tested (60ug/ml).

15

Serum from the patient described in Example 4 was also tested in parallel with normal human serum. As shown in Figures 4 and 5, in absence of either drug the baseline CH50 values of the normal human serum and the serum from the patient serum samples were within the normal human range of >70 CH50 U Eq/ml.

20

Coversin inhibited both normal human serum and serum from the patient with an amino acid substitution at Arg885 equally well. Less than 5% of baseline CH50 (U Eq/ml) was seen at Coversin concentrations of approximately 15ug/ml.

25 Eculizumab inhibited normal human serum at the expected dose, with less than 5% of baseline CH50 (U Eq/ml) being achieved. Akin to the patient serum from Example 2, at doses above 25ug/ml eculizumab inhibited complement activity similarly in normal human serum and serum from the Example 4 patient, but it did not completely inhibit serum from the Example 4 patient, with approximately 10% of baseline CH50 remaining at the highest 30 dose of eculizumab tested (50ug/ml).

Eculizumab does not completely inhibit complement activity in serum from both patients (Example 2 and Example 4) who received no benefit from therapeutic treatment with eculizumab. This supports the hypothesis that complement inhibition in PNH treatment 5 needs to be higher than this to see therapeutic benefit.

Using recombinant expression *Nishimura et al.* (2014) showed that the C5 p.Arg885His polymorphism seen in the Example 2 patient completely ablates eculizumab binding to C5. The partial inhibition of the Example 2 patient's complement serum by eculizumab shown 10 in the current study (Figures 6 and 7) is understandable as the Example 2 patient and all other individuals with the polymorphism identified to date are heterozygotes with a normal copy of C5 and a copy of p.Arg885His C5. If both copies are fully expressed, eculizumab will fully inhibit 50% of the C5 protein present in these individuals. The fact that only 20% residual CH50 activity was seen may reflect the fact that the Example 2 patient was 15 receiving fresh blood products every day which likely increased the ratio of normal C5 to p.Arg885His C5, thus reducing the relative amount of C5 p.Arg885His not inhibited by eculizumab.

Eculizumab appears to inhibit the Example 4 patient's serum to a greater extent than the 20 Example 2 patient's serum, though some residual complement activity remains at even the highest dose of eculizumab. A possible explanation is that the amino acid change at Arg885 is a conservative one that has a less profound effect on eculizumab binding than p.Arg885His.

25 By contrast, Coversin is an equally effective inhibitor of normal human serum, and the serum from the two patients which are not fully inhibited by eculizumab. Complete inhibition by Coversin is understandable as it likely binds to a different site on C5 than eculizumab. Furthermore Coversin has been shown to be an equally effective inhibitor of C5 in a wide range of mammalian species including man, cynomologus monkey, pig, rat, 30 mouse, rabbit and guinea pig. This indicates that binding of Coversin to C5 is far more tolerant of differences in the amino acid sequence of C5 than eculizumab which is only

able to inhibit human C5. Coversin should be considered for treatment of patients that would benefit from C5 inhibition but who gain little or no therapeutic treatment from administration of eculizumab due to polymorphisms in C5 that prevent or reduce the affinity of the binding interaction between eculizumab and C5.

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**CLAIMS:**

1. A method of treating or preventing a complement-mediated disease and/or disorder comprising administering to a subject known to have a complement C5 polymorphism and in need thereof a therapeutically or prophylactically effective amount of an agent that 5 inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway, wherein the agent is
  - a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2 or is a functional equivalent of this protein;
  - b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid 10 sequence according to SEQ ID NO: 2 or is a functional equivalent of this protein; or
  - c) a nucleic acid molecule encoding a protein as recited in (a) or (b);and wherein the complement C5 polymorphism is at the Arg885 residue of wildtype C5 and decreases the effectiveness of eculizumab;
- 15 wherein the functional equivalent of:
  - (a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
  - (b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2; is
    - 20 i. a homologue comprising or consisting of a sequence with at least 80% identity to the sequence of amino acids 19 to 168 or amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
    - ii. a fragment of a protein, wherein the protein has the amino acid sequence according to SEQ ID NO: 2, or a fragment of a homologue, wherein the homologue has at least 80% identity to the sequence of amino acids 19 to 168 or amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2,

wherein said functional equivalent retains the ability to bind complement C5 and to prevent the cleavage of complement C5 by C5 convertase into complement C5a and complement C5b-9.

2. Use of an agent that inhibits the classical complement pathway, the alternative 5 complement pathway and the lectin complement pathway in the manufacture of a medicament for use in a method of treating or preventing a complement-mediated disease and/or disorder in a subject known to have a complement C5 polymorphism, wherein the agent is:

- a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid 10 sequence according to SEQ ID NO: 2 or is a functional equivalent of this protein;
- b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2 or is a functional equivalent of this protein; or
- c) a nucleic acid molecule encoding a protein as recited in a) or b);

15 and wherein the complement C5 polymorphism is at the Arg885 residue of wildtype C5 and decreases the effectiveness of eculizumab;

wherein the functional equivalent of:

- (a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
- 20 (b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2; is
  - i. a homologue comprising or consisting of a sequence with at least 80% identity to the sequence of amino acids 19 to 168 or amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
  - 25 ii. a fragment of a protein, wherein the protein has the amino acid sequence according to SEQ ID NO: 2, or a fragment of a homologue, wherein the homologue has at least 80% identity to the sequence of amino acids 19 to 168 or amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2,

wherein said functional equivalent retains the ability to bind complement C5 and to prevent the cleavage of complement C5 by C5 convertase into complement C5a and complement C5b-9.

3. A method of treating or preventing a complement-mediated disease and/or disorder  
5 comprising the steps of:

- a) identifying a subject with a C5 polymorphism; and
- b) administering to said subject a therapeutically or prophylactically effective amount  
of an agent that inhibits the classical complement pathway, the alternative  
complement pathway and the lectin complement pathway, wherein the agent is:

10 a) a protein comprising or consisting of amino acids 19 to 168 of the amino  
acid sequence according to SEQ ID NO: 2 or is a functional equivalent of  
this protein;

15 b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid  
sequence according to SEQ ID NO: 2 or is a functional equivalent of this  
protein; or

c) a nucleic acid molecule encoding a protein as recited in (a) or (b);

and wherein the complement C5 polymorphism is at the Arg885 residue of wildtype C5  
and decreases the effectiveness of eculizumab;

wherein the functional equivalent of:

20 (a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid  
sequence according to SEQ ID NO: 2; or

(b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid  
sequence according to SEQ ID NO: 2; is

- i. a homologue comprising or consisting of a sequence with at least 80% identity  
25 to the sequence of amino acids 19 to 168 or amino acids 1 to 168 of the amino  
acid sequence according to SEQ ID NO: 2; or
- ii. a fragment of a protein, wherein the protein has the amino acid sequence  
according to SEQ ID NO: 2, or a fragment of a homologue, wherein the  
homologue has at least 80% identity to the sequence of amino acids 19 to 168

or amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2;

and wherein said functional equivalent retains the ability to bind complement C5 and to prevent the cleavage of complement C5 by C5 convertase into complement C5a and 5 complement C5b-9.

4. Use of an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in the manufacture of a medicament for use in a method of treating or preventing a complement-mediated disease and/or disorder, wherein said method comprises the steps of:

- 10 a) identifying a subject with a C5 polymorphism; and
- b) administering to said subject a therapeutically or prophylactically effective amount of an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway, wherein the agent is:
  - i) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2 or is a functional equivalent of this protein;
  - 15 ii) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2 or is a functional equivalent of this protein; or
  - 20 iii) a nucleic acid molecule encoding a protein as recited in i) or ii);

and wherein the complement C5 polymorphism polymorphism is at the Arg885 residue of wildtype C5 and decreases the effectiveness of eculizumab;

wherein the functional equivalent of:

- (a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
- 25 (b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2; is

- i. a homologue comprising or consisting of a sequence with at least 80% identity to the sequence of amino acids 19 to 168 or amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
- ii. a fragment of a protein, wherein the protein has the amino acid sequence according to SEQ ID NO: 2, or a fragment of a homologue, wherein the homologue has at least 80% identity to the sequence of amino acids 19 to 168 or amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2,

and wherein said functional equivalent retains the ability to bind complement C5 and to prevent the cleavage of complement C5 by C5 convertase into complement C5a and complement C5b-9.

5. A method of treating or preventing a complement-mediated disease and/or disorder comprising administering to a subject known to have a complement C5 polymorphism and in need thereof a therapeutically or prophylactically effective amount of an agent that 10 inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway, wherein said agent is administered to a subject on the basis 15 the subject having been determined to have a C5 polymorphism, wherein the agent is:

- a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2 or is a functional equivalent of this protein;
- 20 b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2 or is a functional equivalent of this protein; or
- c) a nucleic acid molecule encoding a protein as recited in a) or b);

and wherein the complement C5 polymorphism is at the Arg885 residue of wildtype C5 25 and decreases the effectiveness of eculizumab;

wherein the functional equivalent of:

- (a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
- (b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2; is

- i. a homologue comprising or consisting of a sequence with at least 80% identity to the sequence of amino acids 19 to 168 or amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
- ii. a fragment of a protein, wherein the protein has the amino acid sequence according to SEQ ID NO: 2, or a fragment of a homologue, wherein the homologue has at least 80% identity to the sequence of amino acids 19 to 168 or amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2,

5 wherein said functional equivalent retains the ability to bind complement C5 and to  
10 prevent the cleavage of complement C5 by C5 convertase into complement C5a and  
complement C5b-9.

6. Use of an agent that inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway in a subject with a C5 polymorphism in the manufacture of a medicament for treating a complement-mediated  
15 disease and/or disorder, wherein said agent is administered to a subject on the basis the subject having been determined to have a C5 polymorphism, wherein the agent is:

- a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2 or is a functional equivalent of this protein;
- b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2 or is a functional equivalent of this protein;  
20 or
- c) a nucleic acid molecule encoding a protein as recited in a) or b);

and wherein the complement C5 polymorphism is at the Arg885 residue of wildtype C5 and decreases the effectiveness of eculizumab;

25 wherein the functional equivalent of:

- (a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
- (b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2; is

- i. a homologue comprising or consisting of a sequence with at least 80% identity to the sequence of amino acids 19 to 168 or amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
- ii. a fragment of a protein, wherein the protein has the amino acid sequence according to SEQ ID NO: 2, or a fragment of a homologue, wherein the homologue has at least 80% identity to the sequence of amino acids 19 to 168 or amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2,

5 wherein said functional equivalent retains the ability to bind complement C5 and to prevent the cleavage of complement C5 by C5 convertase into complement C5a and complement C5b-9.

10 7. A method according to any one of claims 1, 3 or 5 or the use according to any one of claims 2, 4 or 6, wherein the subject is a mammal.

15 8. A method or use according to claim 7, wherein the mammal is a human.

9. A method according to any one of claims 1, 3 or 5 to 8 or the use according to any one of claims 2, 4 or 6 to 8, wherein the subject with a complement C5 polymorphism is identified by:

- a) an unexpectedly poor clinical response to an agent that inhibits one of the complement pathways; and/or
- 20 b) testing the ability of an agent that inhibits one of the complement pathways to inhibit complement activation in the subject; and/or
- c) molecular genetic analysis.

10. A method according to any one of claims 1, 3 or 5 to 9 or the use according to any one of claims 2, 4 or 6 to 9, wherein the complement C5 polymorphism is Arg885Cys.

25 11. A method according to any one of claims 1, 3 or 5 to 9 or the use according to any one of claims 2, 4 or 6 to 9, wherein the complement C5 polymorphism is Arg885His.

12. A method according to any one of claims 1, 3 or 5 to 11 or the use according to any one of claims 2, 4 or 6 to 11, wherein the functional equivalent of:

- a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
- b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2;

5 is a homologue having greater than 90% sequence identity to the protein as defined in a) or b).

13. A method or use according to claim 12, wherein the wherein the functional equivalent of:

- a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
- b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2;

is a homologue having greater than 95% sequence identity to the protein as defined in a) or b).

15 14. A method or use according to claim 12, wherein the wherein the functional equivalent of:

- a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2; or
- b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2;

20 is a homologue having greater than 98% sequence identity to the protein as defined in a) or b).

15. A method or use according to claim 12, wherein the wherein the functional equivalent of:

25 a) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2; or

- b) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2;

is a homologue having greater than 99% sequence identity to the protein as defined in a) or b).

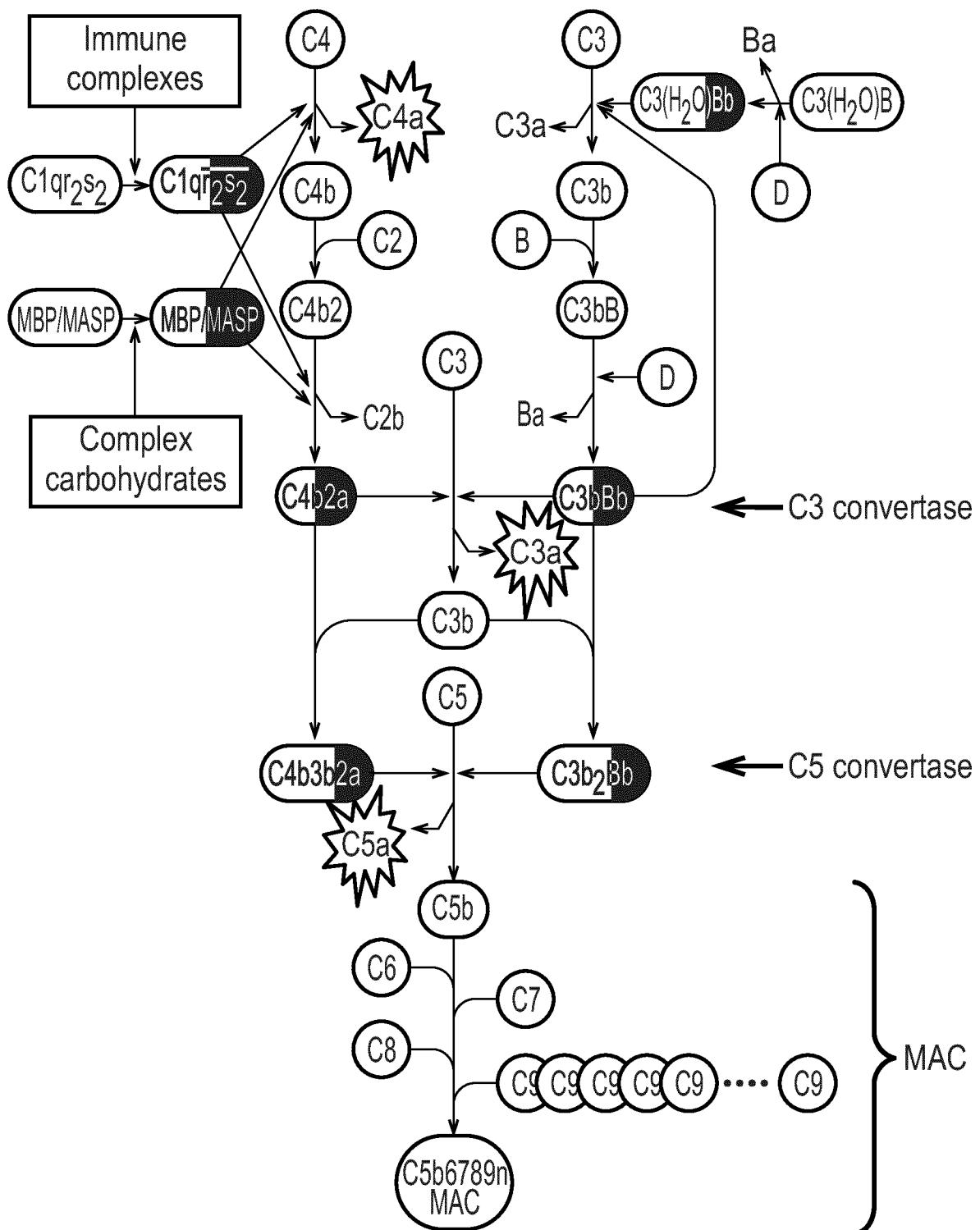
16. A method according to any one of claims 1, 3 or 5 to 15 or the use according to any one of claims 2, 4 or 6 to 15, wherein the complement mediated disease and/or disorder is  
5 selected from acute pancreatitis, age related macular degeneration (AMD), atypical haemolytic uremic syndrome (aHUS), Alzheimer's disease, Huntington's disease, Parkinson's disease, allergic encephalomyelitis, allotransplantation, asthma, adult respiratory distress syndrome, influenza, burn injuries, Crohn's disease, glomerulonephritis, haemolytic anaemia, haemodialysis, hereditary angioedema, ischaemia  
10 reperfusion injuries, multiple system organ failure, multiple sclerosis, myasthenia gravis, myocardial infarction, paroxysmal nocturnal haemoglobinuria (PNH), psoriasis, rheumatoid arthritis, septic shock, systemic lupus erythematosus, stroke, thrombotic thrombocytopaenic purpura (TTP), traumatic brain injury, vascular leak syndrome, transplantation rejection and graft versus host disease (GvHD), peripheral nerve disorders  
15 and respiratory disorders.

17. A method according to any one of claims 1, 3 or 5 to 16 or the use according to any one of claims 2, 4 or 6 to 16, wherein the agent is:

(i) a protein comprising or consisting of amino acids 19 to 168 of the amino acid sequence according to SEQ ID NO: 2;  
20 (ii) a protein comprising or consisting of amino acids 1 to 168 of the amino acid sequence according to SEQ ID NO: 2; or  
(iii) a nucleic acid molecule encoding a protein as recited in (i) or (ii).

18. A method according to any one of claims 1, 3 or 5 to 17 or the use according to any one of claims 2, 4 or 6 to 17, wherein the complement-mediated disease and/or disorder is  
25 selected from paroxysmal nocturnal haemoglobinuria, graft versus host disease, thrombotic thrombocytopaenic purpura and atypical haemolytic uremic syndrome.

FIG. 1

ClassicalAlternative

**FIG. 2**

ATGCTGGTTTGGTACCTGATTTCCTTCTCGAAACATCGCATATGCTGACAGC 60  
 M L V T L I F S A N I A Y A D S 20  
 GAAAGCCACTGCAACTGGAAAGCCTGTTGACCGCTTCCAAGCTTCACTGGCATAA 120  
 E S D C T G S E P V D A F Q A F S E G K 40  
 GAGGCATATGTCCTGGTGGGTGAGGTCCACGGATCCAAAGCGAGGGACTGCTGAAAGGAGAA 180  
 E A Y V L V R S T D P K A R D C L K G E 60  
 CCAGCCGGAGAAAGCAGGACAAACACGGTTGGCATGACGTTAACGAAATGGCACA 240  
 P A G E K Q D N T L P V M M T F K N G T 80  
 GACTGGGGCTCAACCGATTGGACCGTTTACTTTGGACCGGGCAAAGGTAACGCCAACCCCTT 300  
 D W A S T D W T F T L D G A K V T A T L 100  
 GGTAACCTAACCCAAATAAGGGAAGTGGTCTACGACTCGCAAAAGTCATCACTGCCAACCGTT 360  
 G N L T Q N R E V V Y D S Q S H H C H V 120  
 GACAAGGGTGGAGGAAGGAAAGTCCAGATTGAGATGTGGATGCTCGATGCCGGAGGGCTT 420  
 D K V E K E V P D Y E M W M L D A G G L 140  
 GAAGTGGAAAGTGGAGGTGCTGCCGTCAAAAGCTTGAACAGTTGGCGTCTGGCAGGAACCAA 480  
 E V E V E C C R Q K L E E L A S G R N Q 160  
 ATGTATCCCACATCTCAAGGACTGCTAG 507  
 M Y P H L K D C \* 168

FIG 3

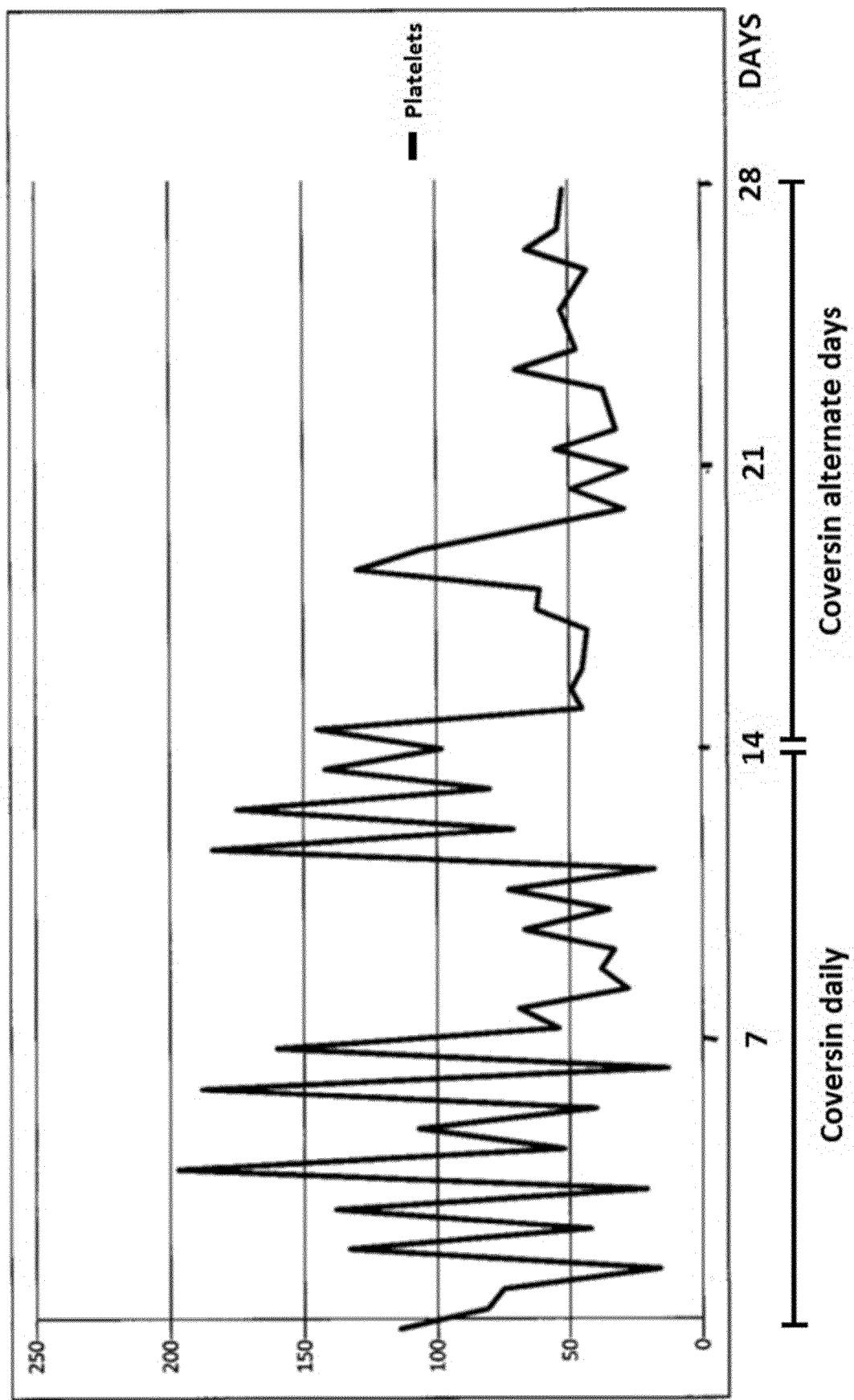


FIG 4

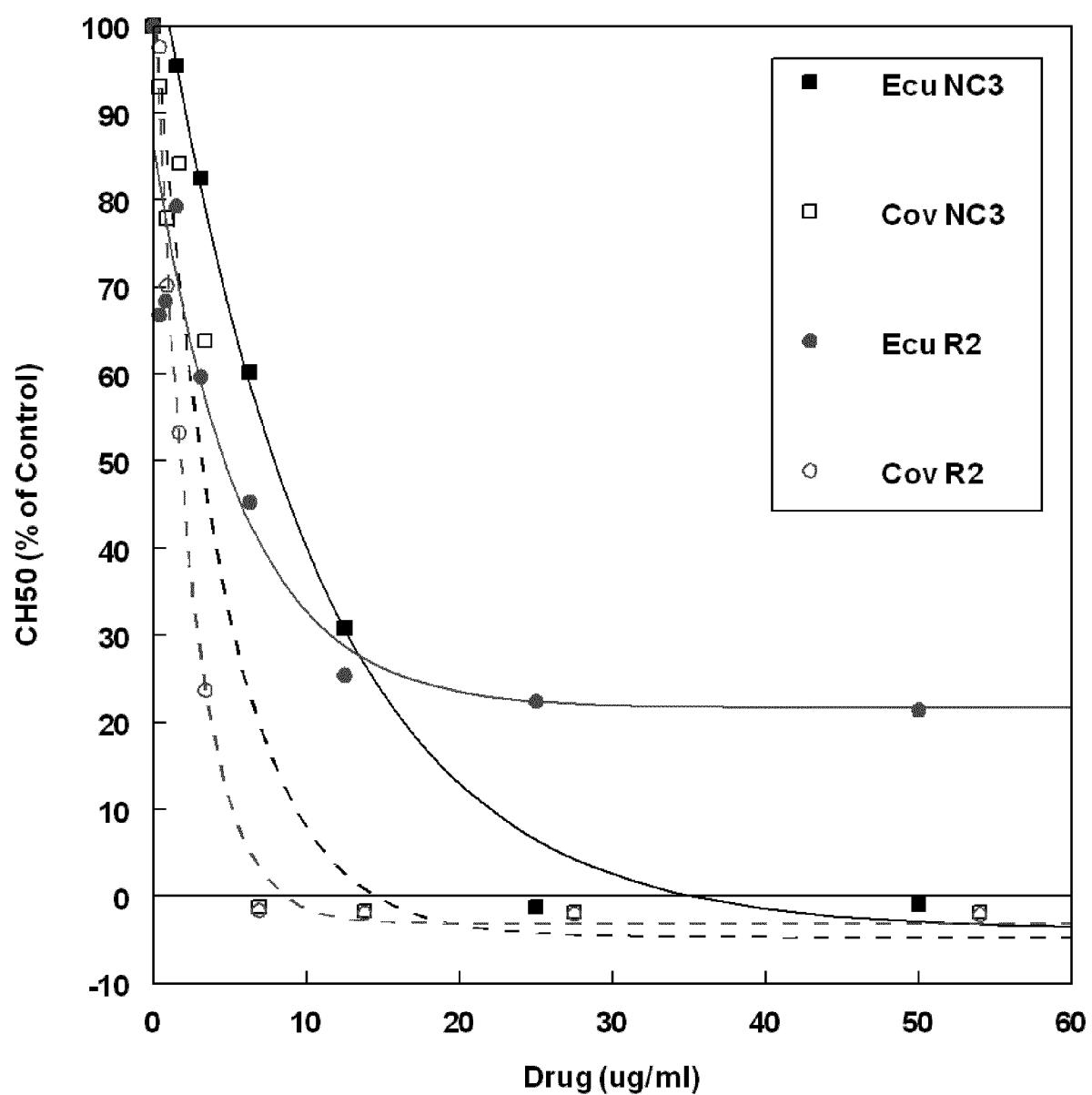


FIG 5

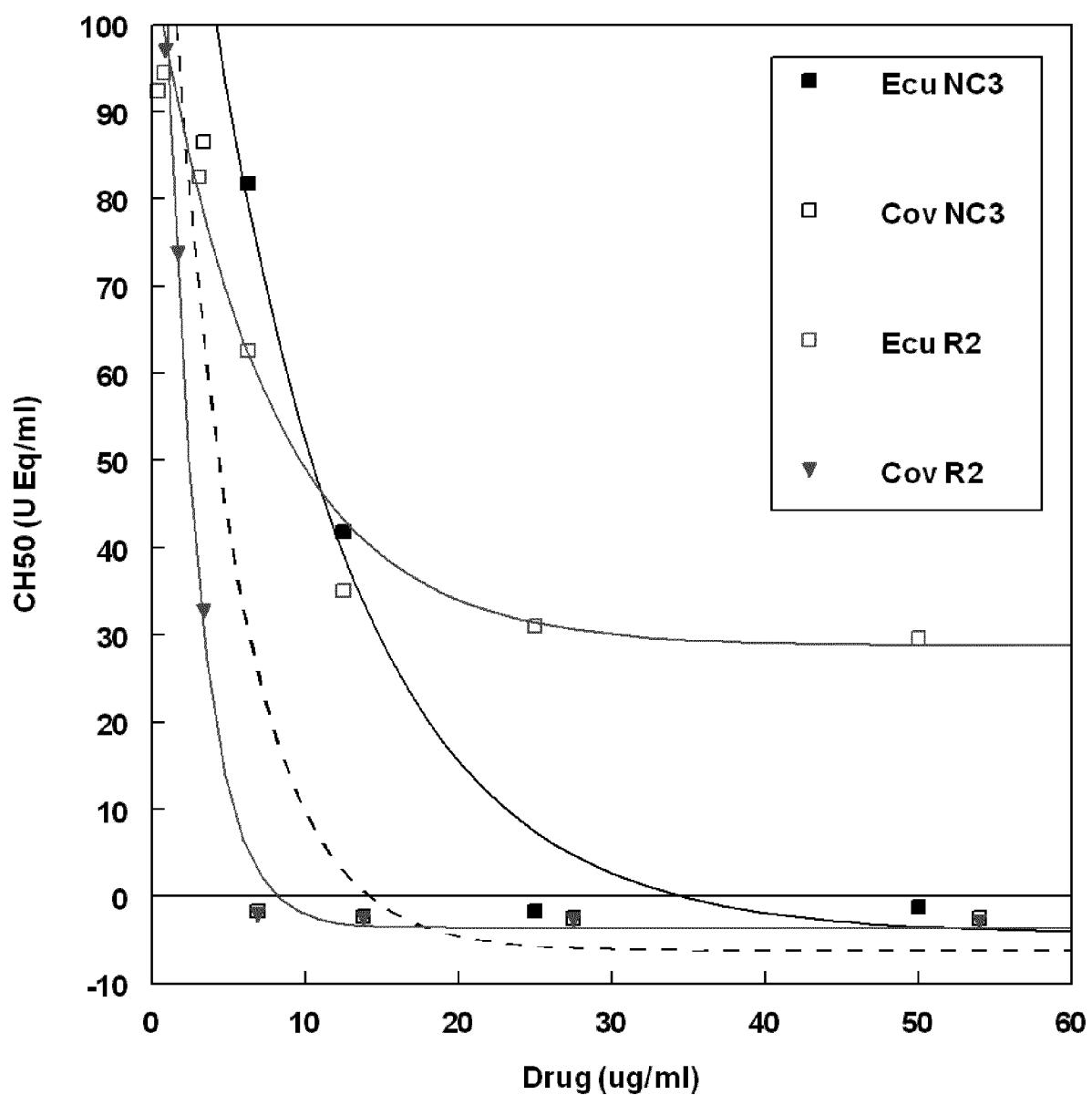


FIG 6

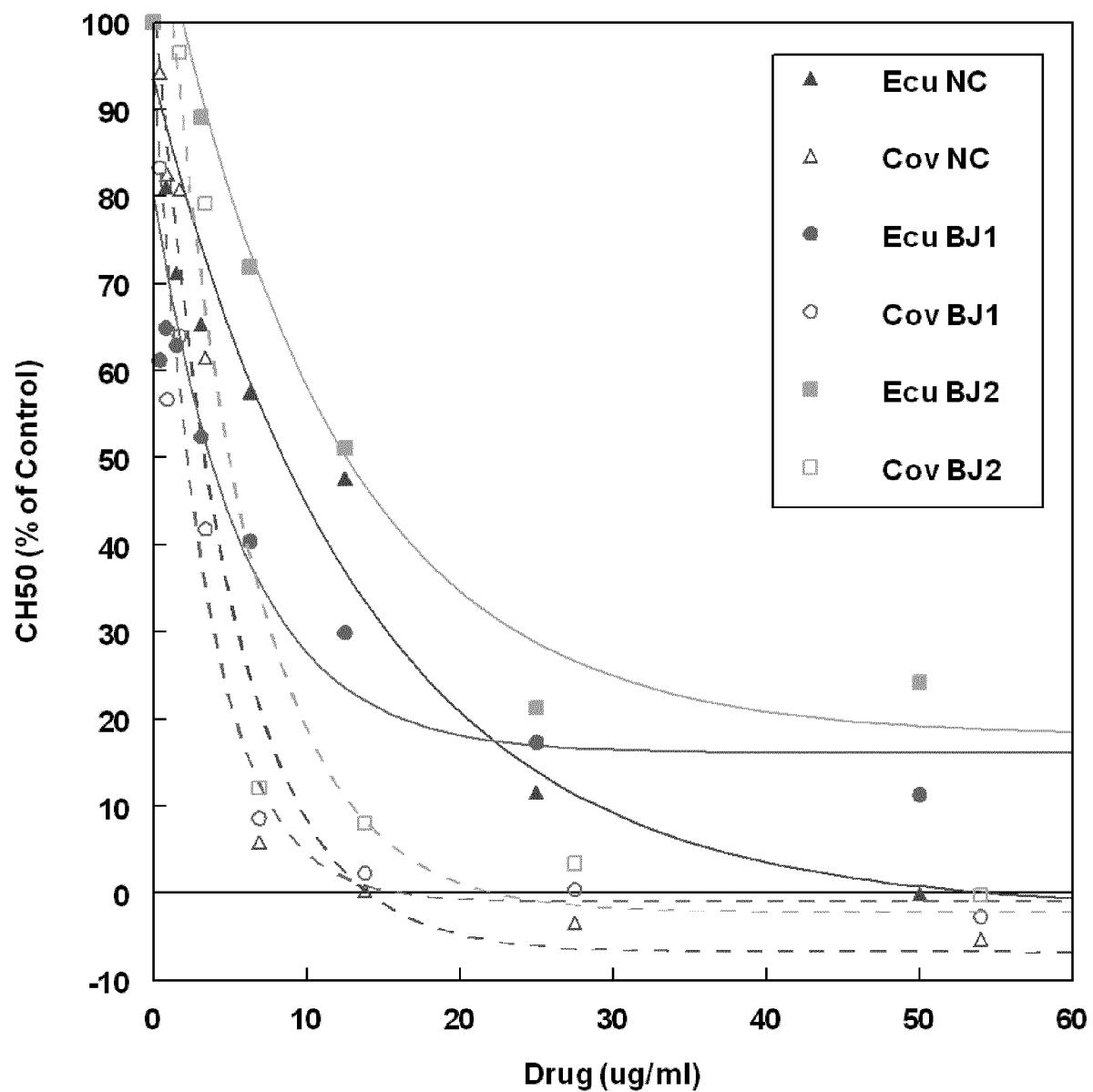


FIG 7

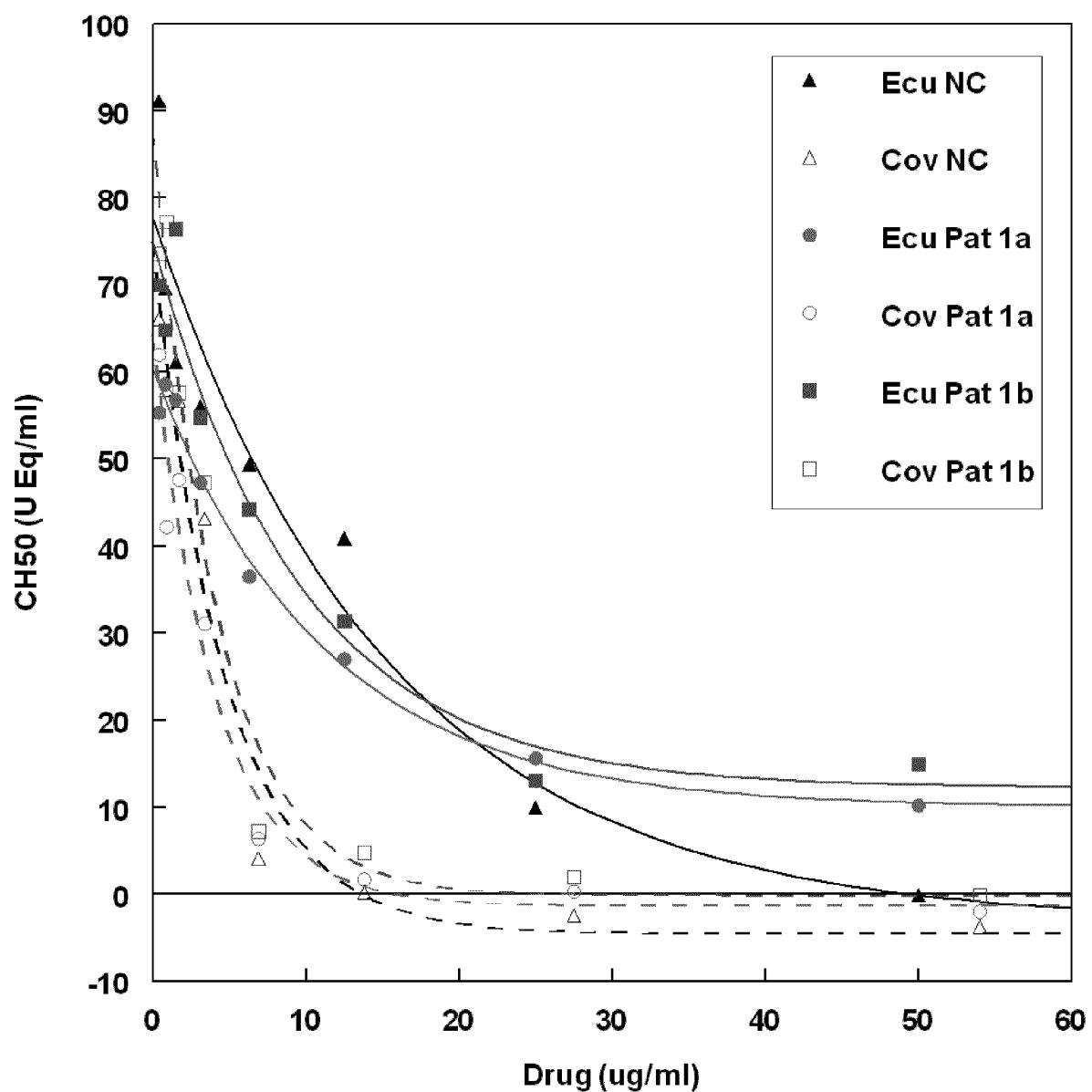
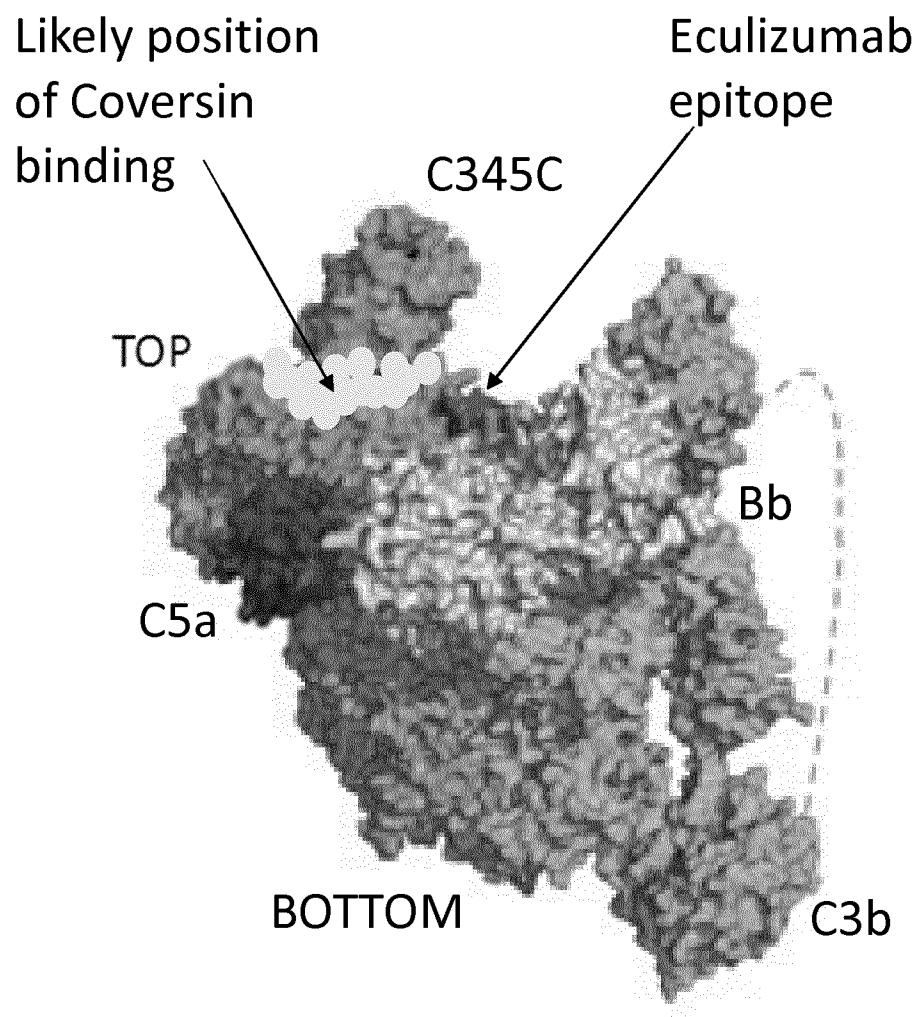


FIG 8



SEQUENCE LISTING

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<213> Ornithodoros moubata

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gaggcatatg tcctggtgag gtccacggat cccaaagcga gggactgctt gaaaggagaa 180

ccagccggag aaaagcagga caacacgtt ccggtgatga tgacgttaa gaatggcaca 240

gactgggctt caaccgattt gacgtttact ttggacggcg caaaggtaac ggcaaccctt 300

ggttaacctaa cccaaaatag ggaagtggtc tacgactcgc aaagtcatca ctgccacgtt 360

gacaaggctcg agaaggaagt tccagattt gagatgtgga tgctcgatgc gggagggctt 420

gaagtggaaag tcgagtgctg ccgtcaaaag cttgaagagt tggcgtctgg caggaaccaa 480

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Tyr Ala Asp Ser Glu Ser Asp Cys Thr Gly Ser Glu Pro Val Asp Ala

20 25 30

Phe Gln Ala Phe Ser Glu Gly Lys Glu Ala Tyr Val Leu Val Arg Ser

35 40 45

Thr Asp Pro Lys Ala Arg Asp Cys Leu Lys Gly Glu Pro Ala Gly Glu

50 55 60

Lys Gln Asp Asn Thr Leu Pro Val Met Met Thr Phe Lys Asn Gly Thr

65 70 75 80

Asp Trp Ala Ser Thr Asp Trp Thr Phe Thr Leu Asp Gly Ala Lys Val

85 90 95

Thr Ala Thr Leu Gly Asn Leu Thr Gln Asn Arg Glu Val Val Tyr Asp

100 105 110

Ser Gln Ser His His Cys His Val Asp Lys Val Glu Lys Glu Val Pro

115 120 125

Asp Tyr Glu Met Trp Met Leu Asp Ala Gly Gly Leu Glu Val Glu Val

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145 150 155 160

Met Tyr Pro His Leu Lys Asp Cys

165

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ggagaaccag ccggagaaaa gcaggacaac acgttgccgg ttagatgac gtttagaat 180

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20 25 30

Pro Lys Ala Arg Asp Cys Leu Lys Gly Glu Pro Ala Gly Glu Lys Gln

35 40 45

Asp Asn Thr Leu Pro Val Met Met Thr Phe Lys Asn Gly Thr Asp Trp

50 55 60

Ala Ser Thr Asp Trp Thr Phe Thr Leu Asp Gly Ala Lys Val Thr Ala

65 70 75 80

Thr Leu Gly Asn Leu Thr Gln Asn Arg Glu Val Val Tyr Asp Ser Gln

85 90 95

Ser His His Cys His Val Asp Lys Val Glu Lys Glu Val Pro Asp Tyr

100 105 110

Glu Met Trp Met Leu Asp Ala Gly Gly Leu Glu Val Glu Val Glu Cys

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20 25 30

Lys Ala Arg Asp Cys Leu Lys Gly Glu Pro Ala Gly Glu Lys Gln Asp

35 40 45

Asn Thr Leu Pro Val Met Met Thr Phe Lys Asn Gly Thr Asp Trp Ala

50 55 60

Ser Thr Asp Trp Thr Phe Thr Leu Asp Gly Ala Lys Val Thr Ala Thr

65 70 75 80

Leu Gly Asn Leu Thr Gln Asn Arg Glu Val Val Tyr Asp Ser Gln Ser

85 90 95

His His Cys His Val Asp Lys Val Glu Lys Glu Val Pro Asp Tyr Glu

100 105 110

Met Trp Met Leu Asp Ala Gly Gly Leu Glu Val Glu Val Glu Cys Cys

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35 40 45

Thr Leu Pro Val Met Met Thr Phe Lys Asn Gly Thr Asp Trp Ala Ser

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Thr Asp Trp Thr Phe Thr Leu Asp Gly Ala Lys Val Thr Ala Thr Leu

65 70 75 80

Gly Asn Leu Thr Gln Asn Arg Glu Val Val Tyr Asp Ser Gln Ser His

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His Cys His Val Asp Lys Val Glu Lys Glu Val Pro Asp Tyr Glu Met

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Trp Met Leu Asp Ala Gly Gly Leu Glu Val Glu Val Glu Cys Cys Arg

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65 70 75 80

Asn Leu Thr Gln Asn Arg Glu Val Val Tyr Asp Ser Gln Ser His His

85 90 95

Cys His Val Asp Lys Val Glu Lys Glu Val Pro Asp Tyr Glu Met Trp

100 105 110

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Lys Leu Glu Glu Leu Ala Ser Gly Arg Asn Gln Met Tyr Pro His Leu

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Asp Cys Leu Lys Gly Glu Pro Ala Gly Glu Lys Gln Asp Asn Thr Leu

35 40 45

Pro Val Met Met Thr Phe Lys Asn Gly Thr Asp Trp Ala Ser Thr Asp

50 55 60

Trp Thr Phe Thr Leu Asp Gly Ala Lys Val Thr Ala Thr Leu Gly Asn

65 70 75 80

Leu Thr Gln Asn Arg Glu Val Val Tyr Asp Ser Gln Ser His His Cys

85 90 95

His Val Asp Lys Val Glu Lys Glu Val Pro Asp Tyr Glu Met Trp Met

100 105 110

Leu Asp Ala Gly Gly Leu Glu Val Glu Val Glu Cys Cys Arg Gln Lys

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gaaaaggcagg acaacacgtt gccggtgatg atgacgttta agaatggcac agactggct 180

tcaaccgatt ggacgttac ttggacggc gcaaaggtaa cggcaaccct tggtaaccta 240

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20 25 30

Cys Leu Lys Gly Glu Pro Ala Gly Glu Lys Gln Asp Asn Thr Leu Pro

35 40 45

Val Met Met Thr Phe Lys Asn Gly Thr Asp Trp Ala Ser Thr Asp Trp

50 55 60

Thr Phe Thr Leu Asp Gly Ala Lys Val Thr Ala Thr Leu Gly Asn Leu

65 70 75 80

Thr Gln Asn Arg Glu Val Val Tyr Asp Ser Gln Ser His His Cys His

85 90 95

Val Asp Lys Val Glu Lys Glu Val Pro Asp Tyr Glu Met Trp Met Leu

100 105 110

Asp Ala Gly Gly Leu Glu Val Glu Val Cys Cys Arg Gln Lys Leu

115 120 125

Glu Glu Leu Ala Ser Gly Arg Asn Gln Met Tyr Pro His Leu Lys Asp

130 135 140

Cys

145