Title: METHOD OF TREATMENT OF COMPLEMENT MEDIATED NEURODEVELOPMENTAL DISORDER SECONDARY TO ZIKA VIRUS INFECTION

Abstract: The present invention relates to methods of treating or preventing complement mediated neurodevelopmental disorders secondary to maternal infection with Zika virus, comprising administering to a pregnant female or to a foetus or embryo, a therapeutically or prophylactically effective amount of an agent which is a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein.
METHOD OF TREATMENT OF COMPLEMENT MEDIATED NEURODEVELOPMENTAL DISORDER SECONDARY TO ZIKA VIRUS INFECTION

FIELD OF THE INVENTION

The present invention relates to methods of treating and preventing complement mediated neurodevelopmental disorders.

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

BACKGROUND TO THE INVENTION

It is recognised that certain human autoimmune diseases of the peripheral nervous system may occur as a consequence of bacterial or viral infections. Such conditions include Guillain Barré syndrome (GBS) occurring after Campylobacter or influenza infection. It has been shown that in such cases the membrane attack complex (MAC), a product of activation of the terminal complement system, is deposited on target tissues such as myelin sheath cells following the local formation of autoantibody-antigen complexes. Deposition of MAC causes destructive cell lysis through membrane pore formation. Similarly Epstein Barr virus, hepatitis E virus and rotavirus, amongst other agents, have been postulated as being involved in myasthenia gravis. [1,2,3]

Alterations in the in utero environment as a result of maternal infection (e.g. viral infection) may have profound and long-term implications for the developing foetus or embryo and in addition to this a substantial proportion of pregnancies worldwide are at risk of such infections during pregnancy. Despite the potential public health implications, little is known about the impact of in utero exposure to such maternal infections on foetal and infant neurological development.

Complement activation, in particular C5, may contribute to neuropathology and adverse outcomes when pregnant females are exposed to malaria [4] and the complement system may be involved in both normal and abnormal neurodevelopmental processes. Complement proteins and their receptors are widely expressed within the central nervous system.

It has previously been considered that the complement mediated neural tissue destruction in these and other autoimmune conditions only affected humans after birth, although it has long been recognised that myasthenia gravis autoantibodies are capable of crossing the placenta from mother to foetus and vice versa [5,6].
Although many infections during pregnancy are benign, certain viral infections during pregnancy can affect multiple organ systems, and some are associated with specific brain damage. For example, microcephaly is associated with congenital varicella syndrome and congenital cytomegalovirus (CMV) primarily affects the ventricle, the organ of Corti and the neurons of the eighth cranial nerve, which explains why it is the leading cause of congenital hearing loss [7].

Microcephaly, a condition in which death of neural progenitor cells in the dorsal telencephalon of the foetus during the first trimester causes a failure of development of the cerebral cortex, is well known to be a sequel of certain viral infections in the mother including rubella, cytomegalovirus, herpes simplex and varicella zoster. Recent pandemics of Zika virus infection in French Polynesia and Brazil have been accompanied by a parallel increase in the number of cases of microcephaly, although a definite causal link has yet to be established [8]. Zika virus is a member of the virus family Flaviviridae and the genus Flavivirus. It is transmitted by daytime-active Aedes mosquitoes, such as A. aegypti and A. albopictus. Although the infection often causes no or only mild symptoms, a 2015 outbreak has reached pandemic levels, with particularly high levels of occurrence in South America. Zika virus is related to dengue, yellow fever, Japanese encephalitis, and West Nile viruses. The illness it causes is similar to a mild form of dengue fever, and there is currently no vaccine.

Ways of treating and preventing complement mediated neurodevelopmental conditions, such as microcephaly, that are secondary to maternal infection by an infectious agent are thus required.

**SUMMARY OF THE INVENTION**

During a maternal infection with an infectious agent, the infectious agent (e.g. the Zika virus or other infectious agents including viruses, bacteria and protozoa), antibodies to such infectious agents, autoantibodies, antibody-antigen complexes, complement pathway components or the MAC complex itself may be capable of crossing the placenta. The infectious agent may also be capable of provoking an antibody response in the foetus or embryo, for instance by causing focal infection of the placenta. According to this invention, any one or more of the above events may give rise to a complement response in the foetus or embryo. Such a response may lead to deposition of the MAC on neural progenitor cells, which may have neurodevelopmental consequences. Therefore, inhibition of complement,
e.g. in the foetus or embryo is seen as a suitable way to treat or prevent these neurodevelopmental consequences.

The present inventors have therefore recognised that administration of the tick protein Coversin (also referred to as EV576 and OmCl in the art and herein [16]) to a pregnant female carrying a foetus or embryo at risk of a complement mediated neurodevelopmental condition secondary to maternal infection by an infectious agent, or its administration to the foetus or embryo itself, may treat or prevent a complement mediated neurodevelopmental condition secondary to maternal infection by an infectious agent.

The invention therefore provides a method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo, secondary to maternal infection by an infectious agent, comprising administering to a pregnant female and/or to a foetus or embryo a therapeutically or prophylactically effective amount of an agent which is a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein.

The invention also provides an agent which is a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein for use in a method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo, secondary to maternal infection by an infectious agent, wherein the agent is administered to the pregnant female and/or to the foetus or embryo.

The invention also provides a method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo, secondary to maternal infection by an infectious agent, comprising administering to a pregnant female and/or foetus or embryo a therapeutically or prophylactically effective amount of an agent which is a nucleic acid molecule encoding a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein.

The invention also provides an agent which is a nucleic acid molecule encoding a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein for use in a method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo, secondary to maternal infection by an infectious agent, wherein the agent is administered to the pregnant female and/or to the foetus or embryo.
In all cases the foetus or embryo may be at risk of a complement mediated neurodevelopmental disorder secondary to maternal infection by an infectious agent, e.g. because of the maternal infection by the infectious agent or because of exposure of the pregnant female to the infectious agent. Therefore an example of a foetus or embryo at risk of a complement mediated neurodevelopmental disorder secondary to maternal infection by an infectious agent is a foetus or embryo wherein the pregnant female (also referred to herein as a “pregnant mother”, irrespective of the outcome of the pregnancy) is infected with an infectious agent, or wherein the pregnant mother has been exposed to an infectious agent. As discussed in more detail below, a foetus or embryo at risk of a complement mediated neurodevelopmental disorder secondary to maternal infection by an infectious agent may itself become infected with the infectious agent, may be exposed to maternal antibodies to the infectious agent, or to maternal autoantibodies produced as a result of the infectious agent, may be exposed to antibody/antigen conjugates arising as a result of the infectious agent, may be exposed to maternal complement proteins, or the results of complement C5 cleavage (e.g. the MAC). Exposure to one or more of the above may lead to MAC deposition on neural progenitor cells. This may be by activation of the complement system in the foetus or embryo (e.g. resulting in the formation of the MAC) or the action of a maternal complement proteins in the foetus or embryo.

Complement

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 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 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 mannan-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 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. C5b initiates the ‘late’ events of complement activation. These comprise a sequence of polymerization reactions in which the terminal complement components interact to form the MAC, which creates a pore in the cell membranes of some pathogens that can lead to their death. The terminal complement components include C5b (initiates assembly of the membrane attack system), C6, C7, C8 and C9.

**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 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 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 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 [9-14].

**Neurodevelopmental disorders**

As discussed above, neurodevelopmental disorders may arise in a foetus or embryo as a result of maternal infection with an infectious agent. Activation of the terminal complement components (either in the mother or in the foetus or embryo), as a result of this infection may give rise to the presence of active MAC complexes. Neurodevelopmental disorders may
arise as a consequence of this, e.g. as a result of deposition of MAC on the foetal or embryonic cells involved in normal neural development (e.g. neural progenitor cells).

Examples of neurodevelopmental disorders have been described including, amongst others, neural tube disorders (such as hydrocephaly, microcephaly, anencephaly, encephalocele (meningocele or meningo(myelo)cele), congenital dermal sinus, spina bifida, spina bifida occulta, myelomeningocele, meningocele, congenital dermal sinus, caudal agenesis), intellectual disability, intellectual developmental disorder, global developmental delay, communication disorders, language disorder, speech sound disorder, childhood-onset fluency disorder (stuttering), social (pragmatic) communication disorder, autism spectrum disorder, attention-deficit/hyperactivity disorder, specific learning disorder, motor disorders, developmental coordination disorder, stereotypic movement disorder, tic disorders. [15]

The invention concerns the treatment and prevention of neurodevelopmental disorders, for example neural tube disorders (such as hydrocephaly, microcephaly, anencephaly and spina bifida). Of particular interest are microcephaly, anencephaly and spina bifida. Microcephaly is of most particular interest.

The invention concerns the treatment and prevention of these neurodevelopmental disorders when they are complement mediated, and secondary to maternal infection by an infectious agent. “Complement mediated” means that the activation of the complement system, has a role in the disorder. For example, the activation of one or more of the complement pathways discussed above may lead to the generation of an active MAC complex which may be deposited on foetal or embryonic cells involved in normal neural development, e.g. neural progenitor cells. Given that the role of the MAC complex in infection is to form transmembrane channels on target cells, which disrupt the cell membrane of target cells, leading to cell lysis and death, the formation of such complexes on cells that are essential for normal neural development, e.g. foetal or embryonic neural progenitor cells, may give rise to neurodevelopmental disorders. The complement mediated neurodevelopmental disorder may thus arise as a result of MAC deposition on cells that are essential for normal neural development, e.g. foetal or embryonic neural progenitor cells.

The complement mediated neurodevelopmental condition is secondary to maternal infection. Maternal infection means that the mother (e.g. a pregnant female) is infected with the infectious agent. Optionally, the infection may be transmitted to the foetus or embryo so that both the mother and the foetus or embryo are infected with the infectious agent.
The embryo or foetus is at risk of a complement mediated neurodevelopmental condition secondary to maternal infection if its mother is infected with the infectious agent or if she has been exposed to the infectious agent. Exposure may have occurred during pregnancy or prior to pregnancy, e.g. up to 1, 2, 3, 4, 5, 6 weeks before fertilization or up to 1, 2, 3, 4 months before fertilization. The risk is particularly associated with the first trimester of pregnancy.

There are several ways in which MAC deposition may occur on developing tissues, e.g. tissues involved in normal neural development such as neural progenitor cells. These include activation of one or more complement pathways in the embryo or foetus as a result of the infectious agent infecting the foetus or embryo, as a result of maternal antibodies to the infectious agent that is infecting the mother crossing the placenta and activating one or more complement pathways in the embryo or foetus, as a result of autoantibodies from the infected mother crossing the placenta and activating one or more complement pathways in the embryo or foetus, as a result of antibody/antigen conjugates from the infected mother crossing the placenta and activating one or more complement pathways in the embryo or foetus resulting in activation of one or more complement pathway in the foetus or embryo, as a result of one or more complement pathway proteins (e.g. C5) crossing the placenta and activating the complement pathway downstream from that protein, or as a result of components of the complement pathway downstream from C5 (e.g. MAC) crossing the placenta.

**Infectious agents**

Infection is the invasion of an organism's body tissues by disease-causing agents, their multiplication, and the reaction of host tissues to these organisms and the toxins they produce. Infections are caused by infectious agents including viruses, viroids, prions, bacteria, nematodes, arthropods, fungi, protozoa, other parasites. It is a host’s ability to fight infections using their innate immune system, specifically the complement system, that gives rise to the possibility of neurodevelopmental defects in a foetus or embryo if a mother is infected with an infectious agent during pregnancy.

**Viruses**

Viruses may be, amongst others, Adeno-associated virus, Aichi virus, Australian bat lyssavirus, BK polyomavirus, Banna virus, Barmah forest virus, Bunyamwera virus, Bunyavirus La Crosse, Bunyavirus snowshoe hare, Cercopithecine herpesvirus, Chandipura

Of particular interest are arboviruses. Of particular interest are viruses in the Flaviviridae family, optionally in the genus Flavivirus.

Specific viruses of interest include Flavivirus family members such as Zika virus, dengue virus, yellow fever virus, Japanese encephalitis virus, and West Nile virus, preferably Zika virus. Other viruses of interest include rubella virus, CMV, HSV, varicella zoster.
In a preferred embodiment therefore the invention therefore provides a method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo, secondary to maternal infection by Zika virus, the method comprising administering to a pregnant female and/or to a foetus or embryo a therapeutically or prophylactically effective amount of an agent as described herein, and the agent as described herein for use in such a method.

**Bacteria, viroids, prions, nematodes, arthropods, fungi, protozoa, other parasites**

The infectious agent may alternatively be a bacteria, viroid, prion, nematode, arthropod, fungi, protozoa or other parasite. Exemplary infectious agents include *Campylobacter*, yeast and *Plasmodium* (e.g. *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* or *P. knowlesi*, preferably *P. falciparum*).

**Outcomes of administration**

The foetus or embryo may be at risk from any complement mediated neurodevelopmental disorder. The outcome of administering the agent of the invention may be e.g. a reduction in the severity of the neurodevelopmental disorder, as discussed in more detail below. This may be manifested for example in an improvement in neurological function and/or measurement of cranial size of the embryo or foetus.

The foetus or embryo at risk of a complement mediated neurodevelopmental disorder may have damage to neural progenitor cells resulting from complement activation, e.g. following maternal infection. As such, the outcome of the treatment of the invention may be a reduction in this neural progenitor cell damage.

The risk to a foetus or newly born child of complement mediated neurodevelopmental disorders can be assessed e.g. by determining whether its mother is infected with an infectious agent, or determining whether the mother has been exposed to an infectious agent, and/or determining whether the embryo or foetus has been infected with the infectious agent. The outcome of the treatment of the invention may be that the neurodevelopmental disorder is prevented (which includes a reduction in severity thereof as discussed below).

The presence of neurodevelopmental disorders can alternatively be assessed *in utero*, e.g. by observing neurodevelopment *in utero* e.g. by detecting the presence or absence or elevated or decreased levels of markers e.g. in a sample from the mother, embryo or foetus or from the amniotic fluid or other appropriate samples, or (where possible) by imaging methods. One
possible marker is C5a. mTOR has also been implicated in neurological diseases. The risk to a foetus or embryo of neurodevelopmental disorders can be assessed by determining whether such neurodevelopmental disorders exist in the foetus or embryo. As such the outcome of the treatment of the invention may be a reduction in the severity of the neurodevelopmental disorder (e.g. an improvement in the prognosis of the foetus or embryo) e.g. as measured by standard methods known in the art.

The above improvements and reductions be assessed before or after birth, and may be relative to the outcome without administration of the agent.

Thus in a further embodiment of the invention, there is provided a method of reducing neural progenitor cell damage in a foetus or embryo, comprising administering to a foetus or embryo at risk thereof or to the pregnant mother a therapeutically or prophylactically effective amount of an agent which is a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein, or which is a nucleic acid molecule encoding the protein or functional equivalent thereof.

There is also provided an agent which is a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein, or which is a nucleic acid molecule encoding the protein or functional equivalent thereof, for use in a method of reducing neural progenitor cell damage in a foetus or embryo, wherein the agent is administered to the foetus or embryo or to the pregnant mother.

Thus in a further embodiment of the invention, there is provided a method of increasing cranial size in a foetus or embryo at risk of developing microcephaly, comprising administering to a pregnant female carrying the foetus or embryo at risk thereof, or to the foetus or embryo a therapeutically or prophylactically effective amount of an agent which is a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein, or which is a nucleic acid molecule encoding the protein or functional equivalent thereof. There is also provided an agent which is a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein, or which is a nucleic acid molecule encoding the protein or functional equivalent thereof, for use in a method of increasing cranial size in a foetus or embryo at risk of developing microcephaly, wherein the agent is administered to a pregnant female carrying the foetus or embryo at risk thereof, or to the foetus or embryo.
Preferred subjects, agents, doses and the like are as disclosed herein. Any reference to any reduction or increase is a reduction or increase compared to said subject in the absence of the treatment. Preferably, the parameter can be quantitated and where this is the case the increase or decrease is preferably statistically significant. For example the increase or decrease may be at least 3, 5, 10, 15, 20, 30, 40, 50% compared to the parameter in the absence of treatment.

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 a pregnant female (e.g. a pregnant woman), a foetus or an embryo. The subject to which the agent is administered is at risk of a complement mediated neurodevelopmental disorder (e.g. a foetus or embryo) or is a pregnant female carrying a foetus or embryo at risk of a complement mediated neurodevelopmental disorder.

For human purposes, the pregnant female may be e.g. at least 10 years old, or at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50 years old, e.g. 10-50, 15-40, 20-30 years old. The terms foetus and embryo are used in accordance with their standard definitions, such that an embryo is defined as the developing pregnancy from the time of fertilization until the end of the eighth week of gestation, when it becomes known as a foetus. The foetus may be less than 42 weeks from the time of fertilization, or less than 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, week(s) from the time of fertilization. The embryo may be less than 9 weeks from the time of fertilization, or less than 8, 7, 6, 5, 4, 3, 2, 1 weeks from the time of fertilization. Of particular relevance are foetuses or embryos in the first trimester (e.g. up to 12 weeks from the time of fertilization), but also up to 13, 14, 15, or 16 weeks (e.g. 2-16, 3-15, 4-14, 5-13, 6-12 weeks from the time of fertilization). This refers to the time at which the treatment is initiated. Equivalent stages of pregnancy can be derived for non-human mammals.

The invention is of relevance to pregnant females carrying a foetus or embryo at risk of a complement mediated neurodevelopmental disorder, as described herein. Preferred such pregnant females may, for example have one or more of the following attributes:

- they may be infected with an infectious agent as defined herein
- they may have been exposed to an infectious agents as defined herein during pregnancy or prior to fertilization e.g. during a time period as also defined elsewhere herein.
Standard techniques known in the art can be used to determine whether a female is pregnant (e.g. hormone tests). Standard techniques known in the art can also be used to determine the stage of the pregnancy, and whether a pregnant female is infected with an infectious agent, e.g. direct detection of the infectious agent. In all cases the foetus or embryo may be at risk of a complement mediated neurodevelopmental disorder secondary to maternal infection by an infectious agent if the mother is infected with an infectious agent or if the mother has been exposed thereto. The infection may be confined to the mother or may have spread to the foetus or embryo.

Methods of the invention may also comprise one or more additional steps of (i) determining whether a female is pregnant, (ii) determining the stage of the pregnancy and (iii) determining whether the mother is infected with an infectious agent or has been exposed to an infectious agent, and (iv) determining whether the foetus or embryo is infected with an infectious agent (e.g. the agent with which the mother is infected).

**Agent to be used in the invention**

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 Coverisin protein, was isolated from the salivary glands of the tick *Ornithodoros moubata*. Coverisin is an outlying member of the lipocalin family and is the first lipocalin family member shown to inhibit complement activation. The Coverisin 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 Coverisin 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 [16], 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 [11], respiratory disorders [12] and peripheral nerve disorders [13]. The ability of 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 [17].

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 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 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 IC50 of approximately 0.02 mg/ml. Functional equivalents of the Coversin protein preferably retain the ability to bind C5 with an IC50 of less than 0.2 mg/ml, preferably less than 0.1 mg/ml, preferably less than 0.05 mg/ml, preferably less than 0.02 mg/ml, preferably less than 1 μg/ml, preferably less than 100 ng/ml, preferably less than 10 ng/ml, more preferably still, less than 1 ng/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.
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. Functional equivalents of the agent also preferably share these properties.

In one aspect of the invention, the agent inhibits the classical complement pathway, the alternative complement pathway and the lectin complement pathway. In one aspect the agent 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. Functional equivalents of the agent also preferably share these properties.

The complement C5 protein, also referred to herein as C5, is cleaved by the C5 convertase enzyme (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 eosinophil chemotaxis, neutrophil activation, increased capillary permeability and inhibition of neutrophil apoptosis [18].

MAC is associated with other important pathological processes including rheumatoid arthritis [19;20], proliferative glomerulonephritis [21], idiopathic membranous nephropathy [22], proteinuria [23], demyelination after acute axonal injury [24] and is also responsible for acute graft rejection following xenotransplantation [25].

Monoclonal antibodies and small molecules that bind and inhibit C5 have been developed to treat various diseases [10], 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 [26]. In contrast, the Coversin agents, and functional equivalents thereof, inhibit complement C5 cleavage both in subjects with wild-type C5 and in subjects with C5 polymorphisms. Examples of C5 polymorphisms include Arg885Cys (encoded by c.2653C>T) and p.Arg885His (encoded by c.2654G>A), both of which decrease the effectiveness of the mAb eculizumab [26]. The term “C5 polymorphism” is thus 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, deletions, single or multiple amino acid substitutions, frame-shifts, truncations and combinations of these changes in the C5 protein.

The ability of an agent to bind C5, including C5 from subjects with C5 polymorphisms, may be determined by standard \textit{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 agent according to the invention binds C5, either wild-type and/or C5 from subjects with C5 polymorphisms, with an IC\textsubscript{50} 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 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, complement activity in the serum can be measured by any means known in the art or described herein.

The agent may also be defined as having the function of inhibiting eicosanoid activity.

The agent according to this aspect of the invention may inhibit leukotrine 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 \textit{in vitro} assays known in the art, for example by means of a competitive ELISA between Coversin and anti-LTB4 antibody competing for binding to labelled LTB4. The agent according to the invention may bind LTB4 with an IC\textsubscript{50} 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. Functional equivalents of the agent also preferably share these properties.

According to one embodiment of the invention, the agent may bind all of C5, C5 from subjects with C5 polymorphisms, and LTB4. The agent according to this embodiment 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.

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*.

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.

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 binding are described in [17].

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. hebraeum*, *Boophilus microplus*, *B. annulatus*, *B. decoloratus*, *Dermacentor reticulatus*, *D. andersoni*, *D. marginatus*, *D. variabilis*, *Haemaphysalis inermis*, *Ha. leachi*, *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.

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 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), 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), Pfam (http://www.sanger.ac.uk/software/pfam), Identify (http://dna.stanford.edu/identify/) 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 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; http://www.ncbi.nlm.nih.gov/) [Blosum 62 matrix; gap open penalty=11 and gap extension penalty=1]. The % identity may be over the full length of the relevant reference sequence (e.g. amino acids 1-168 of SEQ ID NO:2 or amino acids 19-168 of SEQ ID NO:2).

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, or up to 1, 2, 3, 4, 5, 7 or 10 amino acids, (e.g. deletions from the N or C terminus) provided that such mutants retain the ability to bind wild-type C5 and/or C5 from subjects with a C5 polymorphism and/or LTB4. This is relative to the relevant reference sequence (e.g. amino acids 1-168 of SEQ ID NO:2 or amino acids 19-168 of SEQ ID NO:2). 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-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. Fragments may include, for example, polypeptides derived from the Coversin protein sequence (or homologue) which are less than 150 amino acids, less than 145, 140, 135, 130 or 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, polypeptides derived from the Coversin protein sequence (or homologue) which are at least 150 amino acids, at least 145, 140, 135, 130 or 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 LT B4.

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. Preferably such fragment will retain the cysteine spacing referred to above. Fragments with improved properties 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 LT B4 as Coversin. These fragments may be of a size described above for fragments of the Coversin protein.

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 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 Coversin protein and functional equivalents thereof, are preferably in isolated form, e.g. separated from at least one component of the host cell and/or cell growth media in which it was expressed. In some embodiments, the Coversin protein or functional equivalent thereof is purified to at least 90%, 95%, or 99% purity as determined, for example, by electrophoresis or chromatography. 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 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 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, Coversin also possesses the advantage of being easier to administer than other agents such as antibodies like eculizumab.
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 neurodevelopmental disorder. In this context, “treating” includes reducing the severity of the disorder, e.g. if the presence of the disorder is detected before the administration of the agent is commenced.

The term “prophylactically effective amount” used herein refers to the amount of agent needed to prevent the complement-mediated neurodevelopmental disorder. In this context, “preventing” includes reducing the severity of the disorder, e.g. if the presence of the disorder is not detected before the administration of the agent is commenced. Reducing the severity of the disorder could be, for example, reducing the extent or degree of the neural progenitor cell damage, reducing the extent or degree of the neurodevelopmental disorder, and/or improving neurological function and/or the cranial size of the embryo or foetus, as discussed elsewhere herein.

The reduction or improvement is relative to the outcome without administration or the agent as described herein. The outcomes are assessed according to the standard criteria used to assess such patients. To the extent that this can be quantitated, there is a reduction or improvement of at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100% in the relative criteria (e.g. the extent or degree of the neural progenitor cell damage, the extent or degree of the neurodevelopmental disorder, neurological function, or cranial size of the embryo or foetus, as discussed elsewhere herein).

Preferably, the dose of the agent is sufficient to bind as much available C5 as possible in the subject (e.g. the mother and/or the foetus or embryo), 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 may be at least 1x or 1.5 times or twice the molar dose needed to bind all available C5 and/or LTB4 in the subject. The dose of the agent supplied may be e.g. about or at least 1, 1.5, 2, 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 (TCA) 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.

Where a dose is given, this relates to a dose of the agent which is a protein or functional equivalent thereof. Appropriate doses for an agent which is a nucleic acid molecule may be used to give rise to these levels.

Terminal complement activity can be measured by standard assays known in the art, e.g. using the Quidel CH50 haemolysis assay and the sheep red blood cell lytic assay.

The frequency with which the dose needs to be administered will depend on the half-life of the agent involved. The Coversin protein or a functional equivalent thereof, 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 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 may be a lower dose that is repeated at regular intervals. The maintenance dose may be repeated at intervals, such as every 3, 4, 6, 8, 12, 24, or 48 hours. The precise regimen can be determined by routine experimentation, but may ultimately lie with the judgement of the clinician. The maintenance dose may be at least 20, 30, 40, 50, 60, 70, 80, 90 or 100% of the initial loading dose, or up to 20, 30, 40, 50, 60, 70, 80, 90 or 100% of the initial loading dose.

In a further embodiment the same dose is used throughout the course of treatment (e.g. daily).

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 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; 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 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 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 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, 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 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 [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 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 to a pregnant subject. 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 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 maternally 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).
Alternatively the agent is delivered to the embryo or foetus, e.g. by intra-amniotic injection or direct injection into the embryo or foetus, e.g. once the foetal circulation had been established. Preferred doses are as set out above.

In a further alternative the agent is delivered to both the mother and the embryo or foetus, e.g. using the administration routes and doses described in more detail herein.

Preferably the course of treatment is continued for at least 1, 2, 3, 4, 5 or 6 weeks, e.g. at least 7, 8, 9, 10, 15, 20, 25, 30, 35, 40 weeks or until the birth of the baby. Preferably the course of treatment is continued until the first trimester, or until 12, 13, 14, 15, 16, 17, 18, 19, 20 weeks after fertilization.

The course of treatment is preferably continued until the foetus or embryo is no longer considered to be suffering from or at risk of a complement mediated neurodevelopmental disorder, or until the foetus or embryo no longer requires treatment. The course of treatment may thus be administration of the agent (e.g. daily) for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40 weeks or until the birth of the baby. Daily administration may be continued for at least 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks. The frequency of administration may be modified after at least 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks, e.g. to once every two days.

The maintenance dose (e.g. a single daily maintenance dose) may remain constant throughout the course of treatment or the maintenance dose (e.g. a daily maintenance dose) may be modified (e.g. increased or decreased) during the course of treatment. The maintenance dose may be modified in order to maintain terminal complement activity at a desired level, e.g. 10% or less compared to serum from said patient in the absence of treatment or compared to normal control serum. The or each maintenance dose may be continued for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks, e.g. daily for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks. The maintenance dose may be decreased as the subject’s symptoms improve. The amount of agent or the frequency with which the agent is administered may be decreased as the subject’s symptoms improve.

There may thus be an initial loading dose, followed by an initial maintenance dose (e.g. a daily initial maintenance dose) which may be a maintenance dose as defined above, and one or more further maintenance doses (e.g. a daily further maintenance dose), e.g. at least 2, 3, 4, 5 further maintenance doses.

The invention thus further comprises a method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo, secondary to maternal
infection with an infectious agent, comprising administering to the pregnant mother and/or to the embryo or foetus an initial loading dose of the agent as defined above, and then administering maintenance doses (e.g. daily maintenance doses) of the agent as defined above, wherein there is an initial maintenance dose and one or more further maintenance doses.

The invention thus further comprises an agent as defined above for use in a method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo, secondary to maternal infection with an infectious agent, the method comprising administering to the pregnant mother and/or to the foetus or embryo an initial loading dose of the agent as defined above, and then administering maintenance doses (e.g. daily maintenance doses) of the agent as defined above, wherein there is an initial maintenance dose and one or more further maintenance doses.

The one or more further maintenance doses may be determined by testing the terminal complement activity in the pregnant mother or embryo or foetus (e.g. in a biological sample from the pregnant mother or foetus), and determining the further maintenance dose on the basis of the level of terminal complement activity and/or testing the embryo or foetus’ symptoms and determining the further maintenance dose on the basis of the symptoms. The method may optionally further comprise administering said further maintenance dose. Said further dose may be calculated to be at a level that maintains terminal complement activity at the desired level.

Where a biological sample is taken, this may be blood, e.g. a whole blood or a serum sample, e.g. from the mother or from the foetus or embryo. Alternatively it may be amniotic fluid. The method optionally further comprises the step of taking the sample, and further optionally comprises the step of determining the TCA of the sample.

The one or more further maintenance doses may be determined by testing the terminal complement activity in the mother and/or foetus or embryo (e.g. in a biological sample), and determining the further maintenance dose on the basis of the level of terminal complement activity and/or testing the foetus or embryo’s symptoms and determining the further maintenance dose on the basis of the symptoms. The method may optionally further comprise administering said further maintenance dose. Said further dose may be calculated to be at a level that maintains terminal complement activity at the desired level.
In certain aspects, the desired complement activity level is 10% or less compared to serum from said subject in the absence of treatment or compared to normal control serum.

In certain aspects, if the TCA is higher than the desired level the maintenance dose is increased, and optionally wherein if TCA is less than 5, 4, 3, 2, 1% the dose is maintained or decreased.

In certain aspects, if the symptoms deteriorate the maintenance dose is increased, and optionally wherein if the symptoms improve the dose is maintained or decreased.

In some embodiments the mother and/or foetus or embryo is tested within one month of initiating the treatment, within two weeks of initiating the treatment, within a week of initiating the treatment. In other embodiments the mother and/or foetus or embryo is tested once a day or at least once a day, once a week, or at least once a week, once every two weeks or at least once every two weeks, once a month or once every two months.

Preferably the loading dose is 0.57mg/kg or about 0.57mg/kg protein or functional equivalent and the maintenance dose is at least 0.15mg/kg (e.g. at least 0.2mg/kg, 0.15-0.6, 0.2-0.5 or 0.25-0.45mg/kg) or is up to 0.13mg/kg (e.g. up to 0.1mg/kg, 0.05-0.13, 0.075-0.1mg/kg) and optionally (i) that maintenance dose is continued for at least 2, 3, 4, 5, 6 weeks and/or (ii) treatment is continued for at least 6 weeks and/or (iii) treatment is continued daily for at least 3, 4, 5, 6 weeks.

Preferably the loading dose is 0.4-0.6 mg/kg protein or functional equivalent and the maintenance dose is 0.1-0.2 mg/kg, e.g. about 0.14mg/kg, and (i) that maintenance dose is continued for at least 2, 3, 4, 5, 6 weeks and/or (ii) treatment is continued for at least 6 weeks and/or (iii) treatment is continued daily for at least 3, 4, 5, 6 weeks.

In preferred embodiments there is systemic administration of the agents. This means that the agent is present in the circulatory system so that the entire body is affected. This is in contrast with topical administration where the effect is generally local. The systemic presence of the agent can be determined functionally, e.g. on the basis of TCA in the serum of the subject, or directly (e.g. by ELISA or mass spectrometry).

The agent may be administered alone or as part of a treatment regimen also involving the administration of other drugs currently used in the treatment or prophylaxis of patients with infectious agents (e.g. viruses) that may increase risk of a complement mediated neurodevelopmental disorder in foetuses and embryos, such as Zika virus.
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).

**Embodyments of the invention include:**

1. A method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo secondary to maternal infection by an infectious agent, comprising administering to a pregnant female and/or to the foetus or embryo a therapeutically or prophylactically effective amount of an agent which is a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein.

2. An agent which is a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein for use in a method of treating or preventing a complement mediated neurodevelopmental disorder secondary to maternal infection by an infectious agent in a foetus or embryo, wherein the agent is administered to the pregnant female and/or to the foetus or embryo.

3. A method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo secondary to maternal infection by an infectious agent, comprising administering to a pregnant female and/or to the foetus or embryo a therapeutically or prophylactically effective amount of an agent which is a nucleic acid molecule encoding a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein.

4. An agent which is a nucleic acid molecule encoding a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein for use in a method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo secondary to maternal infection by an infectious agent, wherein said agent is administered to the pregnant female and/or to the foetus or embryo.

5. The method of any one of embodiments 1 or 3 or the agent for use of any one of embodiments 2 or 4, wherein the the pregnant female is infected with or has been exposed to an infectious agent.
6. The method or agent for use of embodiment 5 wherein the infectious agent is a Flavivirus.

7. The method or agent for use of embodiment 5 or 6 wherein the infectious agent is selected from Zika virus, dengue virus, yellow fever virus, Japanese encephalitis virus, and West Nile virus.

8. The method or agent for use of any one of embodiments 5 to 7, wherein the infectious agent is Zika virus.

9. The method of any one of embodiments 1, 3 or 5 to 8, or the agent for use of any one of embodiments 2, 4 or 5 to 8, wherein the pregnant female is in the first trimester of pregnancy.

10. The method of any one of embodiments 1, 3, or 5 to 9 or the agent for use of any one of embodiments 2, 4 or 5 to 9 wherein the treatment is continued

   (i) for at least 4 weeks, and/or

   (ii) until the end of the first trimester of pregnancy.

11. The method of any one of embodiments 1, 3, or 5 to 10 or the agent for use of any one of embodiments 2, 4 or 5 to 10 wherein the agent is administered to the pregnant female subcutaneously.

12. The method of any one of embodiments 1, 3, or 5 to 11 or the agent for use of any one of embodiments 2, 4 or 5 to 11 wherein the agent is administered to the foetus or embryo by intra-amniotic injection or direct injection into the embryo or foetus.

13. The method of any one of embodiments 1, 3, or 5 to 12 or the agent for use of any one of embodiments 2, 4 or 5 to 12, wherein the subject is a human.

14. The method of any one of embodiments 1, 3, or 5 to 13 or the agent for use of any one of embodiments 2, 4 or 5 to 13, wherein the agent is administered in a dose sufficient to bind as much available C5 and/or LTB4 as possible in the mother and/or the embryo or foetus, preferably, all available C5.

15. The method of any one of embodiments 1, 3, or 5 to 14 or the agent for use of any one of embodiments 2, 4 or 5 to 14, wherein the agent is administered in a dose 1.5 the molar dose needed to bind all available C5 and/or LTB4 in the mother and/or the embryo or foetus.
16. The method of any one of embodiments 1, 3, or 5 to 15 or the agent for use of any one of embodiments 2, 4 or 5 to 15, wherein the method comprises administering to the pregnant female and/or to the foetus or embryo an initial loading dose of the agent and then administering maintenance doses thereof.

17. The method of embodiment 17 wherein there in an initial maintenance dose and one or more further maintenance doses.

18. The method of any one of embodiments 1, 3, or 5 to 17 or the agent for use of any one of embodiments 2, 4 or 5 to 17, wherein the method further comprises one or more of

(i) determining whether a female is pregnant,

(ii) determining the stage of the pregnancy

(iii) determining whether the mother is infected with an infectious agent or has been exposed to an infectious agent, and

(iv) determining whether the foetus or embryo is infected with an infectious agent.

19. The method of any one of embodiments 1, 3, or 5 to 18 or the agent for use of any one of embodiments 2, 4 or 5 to 18, wherein the agent is administered simultaneously, sequentially or separately with another drug used in the treatment or prophylaxis of the infectious agent.
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 bold type. Nucleotide and amino acid number indicated at right.
REFERENCES:

[16] WO 2004/106369
CLAIMS:

1. A method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo secondary to maternal infection by an infectious agent, comprising administering to a pregnant female and/or to the foetus or embryo a therapeutically or prophylactically effective amount of an agent which is a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein, wherein the infectious agent is Zika virus.

2. An agent which is a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein for use in a method of treating or preventing a complement mediated neurodevelopmental disorder secondary to maternal infection by an infectious agent in a foetus or embryo, wherein the agent is administered to the pregnant female and/or to the foetus or embryo, wherein the infectious agent is Zika virus.

3. A method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo secondary to maternal infection by an infectious agent, comprising administering to a pregnant female and/or to the foetus or embryo a therapeutically or prophylactically effective amount of an agent which is a nucleic acid molecule encoding a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein, wherein the infectious agent is Zika virus.

4. An agent which is a nucleic acid molecule encoding a protein comprising amino acids 19 to 168 of the amino acid sequence in Figure 2 (SEQ ID NO: 2) or a functional equivalent of this protein for use in a method of treating or preventing a complement mediated neurodevelopmental disorder in a foetus or embryo secondary to maternal infection by an infectious agent, wherein said agent is administered to the pregnant female and/or to the foetus or embryo, wherein the infectious agent is Zika virus.

5. The method of any one of claims 1 or 3 or the agent for use of any one of claims 2 or 4, wherein the the pregnant female is infected with or has been exposed to an infectious agent, wherein the infectious agent is Zika virus.
6. The method of any one of claims 1, 3 or 5 or the agent for use of any one of claims 2, 4 or 5, wherein the agent is, or encodes, a protein comprising a sequence having at least 90% sequence identity to the sequence of amino acids 19 to 168 of SEQ ID NO: 2, and said agent binds C5 to prevent the cleavage of complement C5 by convertase into complement C5a and complement C5b-9.

7. The method of any one of claims 1, 3, 5 or 6 or the agent for use of any one of claims 2, 4, 5 or 6, wherein the agent is, or encodes, a protein comprising a sequence having at least 95% sequence identity to the sequence of amino acids 19 to 168 of SEQ ID NO: 2, and said agent binds C5 to prevent the cleavage of complement C5 by convertase into complement C5a and complement C5b-9.

8. The method of any one of claims 1, 3 or 5 to 7, or the agent for use of any one of claims 2, 4 or 5 to 7, wherein the agent is, or encodes, a protein comprising or consisting of the sequence of amino acids 19 to 168 of SEQ ID NO: 2.

9. The method of any one of claims 1, 3 or 5, or the agent for use of any one of claims 2, 4 or 5, wherein agent is, or encodes, a protein comprising the sequence of amino acids 19 to 168 of SEQ ID NO: 2, in which up to 10 amino acid substitutions, insertions or deletions have been made, and the agent binds C5 to prevent the cleavage of complement C5 by convertase into complement C5a and complement C5b-9.

10. The method of any one of claims 1, 3 or 5, or the agent for use of any one of claims 2, 4 or 5, wherein the agent is, or encodes, a fragment of the protein as defined in any of claims 6 to 9, and the agent binds C5 to prevent the cleavage of complement C5 by convertase into complement C5a and complement C5b-9.

11. The method of any one of claims 1, 3 or 5 to 10, or the agent for use of any one of claims 2, 4 or 5 to 10, wherein the pregnant female is in the first trimester of pregnancy.

12. The method of any one of claims 1, 3, or 5 to 11 or the agent for use of any one of claims 2, 4 or 5 to 11 wherein the treatment is continued (i) for at least 4 weeks, and/or (ii) until the end of the first trimester of pregnancy.
13. The method of any one of claims 1, 3, or 5 to 12 or the agent for use of any one of claims 2, 4 or 5 to 12 wherein the agent is administered to the pregnant female subcutaneously.

14. The method of any one of claims 1, 3, or 5 to 13 or the agent for use of any one of claims 2, 4 or 5 to 13, wherein the agent is administered to the foetus or embryo by intra-amniotic injection or direct injection into the embryo or foetus.

15. The method of any one of claims 1, 3, or 5 to 14 or the agent for use of any one of claims 2, 4 or 5 to 14, wherein the subject is a human.

16. The method of any one of claims 1, 3, or 5 to 15 or the agent for use of any one of claims 2, 4 or 5 to 15, wherein the agent is administered in a dose sufficient to bind as much available C5 and/or LTB4 as possible in the mother and/or the embryo or foetus, preferably, all available C5.

17. The method of any one of claims 1, 3, or 5 to 16 or the agent for use of any one of claims 2, 4 or 5 to 16, wherein the agent is administered in a dose 1.5 the molar dose needed to bind all available C5 and/or LTB4 in the mother and/or the embryo or foetus.

18. The method of any one of claims 1, 3, or 5 to 17 or the agent for use of any one of claims 2, 4 or 5 to 17, wherein the method comprises administering to the pregnant female and/or to the foetus or embryo an initial loading dose of the agent and then administering maintenance doses thereof.

19. The method of claim 18 wherein there in an initial maintenance dose and one or more further maintenance doses.

20. The method of any one of claims 1, 3, or 5 to 19 or the agent for use of any one of claims 2, 4 or 5 to 19, wherein the method further comprises one or more of

(i) determining whether a female is pregnant,

(ii) determining the stage of the pregnancy

(iii) determining whether the mother is infected with an infectious agent or has been exposed to an infectious agent, and

(iv) determining whether the foetus or embryo is infected with an infectious agent.
21. The method of any one of claims 1, 3, or 5 to 20 or the agent for use of any one of claims 2, 4 or 5 to 20, wherein the agent is administered simultaneously, sequentially or separately with another drug used in the treatment or prophylaxis of the infectious agent.
FIG. 2

ATGCTGGTTTTGCTGACCCCTGATTCTCCTTTCTCCTTCTCCTTCTCGAGAACATCAGCATATGCTGACAGC 60
MLVTVTLLFSFSANIAYAD 20
GAAAGCGACTGCACCTGGAAGGCAACCTGTTGACGCTTTCCAAGCTTTCAGTGAGGGAANAA 120
ESEDCTGSBEPVDADFQAFSSEGK 40
GAGGCATATGTCCTGTGGAGGGTCCACGGATCCCCAAAGCGAGGGACTGCTTGAAGAGGAA 180
EAYVLPVRSKPDKARDCLKGE 60
CCAGCCGGAAGAAAAGCAGGACAACACGTTGCCGCTGTGATGTACGGCTTTAAAGATAGGCACA 240
PAGEKQDNTLPVMNTFKNVTG 80
GACTGGGCTTCAACGGATTGGACGTTTACTTGGACGCGCCGCAAAGGTACCGCACCCTT 300
DWASTDWTFTLDGAKVTATL 100
GGTAAACCTAAAACCACAAATAGGAAGTTGTTACGACTCCGGAAATGCACCATCGACCTCGAAGCTT 360
GNLTQNREVYDSQHSCHVG 120
GACAAGTCTGAGAAGAAGTTCCAGATTATGAGATGTTGTGGGCTCGATGCAGGGAGGGCTT 420
DKVEKEVPDEMWMDLAGGL 140
GAAAGTGGAGATCGAGTGCGCTCCCGTCAAAAAAGCGGGAGTTGGCCTGCGACGGAACCA 480
EVECRCRQKLEELASGRNQ 160
ATGTATCCCCATCTCAAGGACTGCTAG 507
MYPHLKDC * 168
## INTERNAL SEARCH REPORT

**International application No:** PCT/EP2017/053714

### A. CLASSIFICATION OF SUBJECT MATTER

**INV.** A61K38/17  A61P31/12  
**ADD.**

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

### B. FIELDS SEARCHED

**Minimum documentation searched (classification system followed by classification symbols):**

A61K  A61P

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

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

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
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<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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| X         | WO 2015/185760 A1 (VOLUTION IMMUNO PHARMACEUTICALS SA [CH])  
10 December 2015 (2015-12-10)  
page 12, line 25 - page 14, line 14; claims ----- | 1-21 |

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