

COMPLEMENT FACTOR I AND COMPLEMENT FACTOR I COFACTOR, VECTORS ENCODING THEREFOR AND THERAPEUTIC USE

FIELD OF THE INVENTION

The present invention relates to agents for use in gene therapy. In particular, the invention relates to combinations of Complement Factor I (CFI) and CFI cofactors, such as Complement Factor H-like Protein 1 (FHL1), polynucleotides encoding therefor, and their uses in the treatment or prevention of complement-mediated and complement-associated disorders, including complement-mediated eye diseases, such as age-related macular degeneration (AMD).

10 BACKGROUND TO THE INVENTION

The macula is a small area in the retina of the eye, approximately 3 to 5 millimetres in size, adjacent to the optic nerve. It is the most sensitive area of the retina and contains the fovea, a depressed region that allows for high visual acuity and contains a dense concentration of cones, the photoreceptors that are responsible for colour vision.

15 Age-related macular degeneration (AMD) is the most common cause of functional blindness in developed countries for persons over 50 years of age (Seddon, J.M., Epidemiology of age-related macular degeneration. In: Ogden, T.E., et al., eds. Ryan S.J., ed-in-chief. Retina Vol II. 3rd ed. St. Louis, Mo.: Mosby; 2001: 1039-1050). AMD is associated with neovascularisation originating from the choroidal vasculature and extending into the subretinal space. In addition, AMD is characterised by progressive degeneration of the retina, retinal pigment epithelium (RPE), and underlying choroid (the highly vascular tissue that lies beneath the RPE, between the retina and the sclera).

A variety of factors including oxidative stress, inflammation with a possible autoimmune component, genetic background (e.g. mutations), and environmental or behavioural factors such as smoking and diet may contribute to the pathogenesis of AMD.

The clinical progression of AMD is characterised in stages according to changes in the macula. The hallmark of early AMD is the appearance of drusen, which are accumulations of extracellular debris underneath the retina and appear as yellow spots in the retina during clinical examination and on fundus photographs. Drusen are categorised by size as small (<63 µm), medium (63-124 µm) and large (>124 µm). They are also considered as hard or soft depending on the appearance of their margins on ophthalmological examination. While hard drusen have clearly defined margins, soft drusen have less defined, fluid margins. The

Age-related Eye Disease Study (AREDS) fundus photographic severity scale is one of the main classification systems used for this condition.

Intermediate AMD is diagnosed by large drusen and/or any retinal pigment abnormalities. Intermediate AMD may cause some vision loss, but, like early AMD, it is usually asymptomatic.

Late-stage AMD has been classified into “dry” and “wet” (exudative or neovascular) forms. Dry AMD is more common than wet AMD, but the dry form can progress to the wet form, and the two occur simultaneously in a significant number of cases. Dry AMD is typically characterised by progressive apoptosis of cells in the RPE layer and overlying photoreceptor cells, and frequently also the underlying cells in the choroidal capillary layer. Confluent areas of RPE cell death accompanied by overlying photoreceptor atrophy are referred to as geographic atrophy (GA). Patients with this form of AMD (advanced dry form) experience a slow and progressive deterioration in central vision.

Wet AMD is characterised by bleeding and/or leakage of fluid from abnormal vessels that have grown from the choroidal vessels (choriocapillaris) beneath the RPE and the macula, which can be responsible for sudden and disabling loss of vision. It has been estimated that much of the vision loss that patients experience is due to such choroidal neovascularisation (CNV) and its secondary complications. A subtype of neovascular AMD is termed retinal angiomatous proliferation (RAP). Here, angiomatous proliferation originates from the retina and extends posteriorly into the subretinal space, eventually communicating in some cases with choroidal new vessels.

The complement system (CS) has been implicated in early AMD pathogenesis based on the identification of CS components in drusen from eyes of AMD patients. In AMD, at least 129 types of drusen-deposited proteins have been identified, including different apolipoprotein types (E, B or A-I), several amyloid peptides (P, A β or SA-1), TIMP-3, serum albumin, and certain proteins associated with cellular function (e.g. ATP synthase β subunit, scavenger receptor B2 and retinol dehydrogenase). AMD-derived drusen also contain almost all of the complement proteins, including regulatory proteins (CFH, complement receptor 1 (CR1), vitronectin and clusterin), the products of CS activation and degradation (C1q, C3, C3a, C3b and C5a), and members of the terminal CS pathway comprising the MAC components (i.e. 5, 6, 8 (α , β and γ) and 9) in the separated and complex form. Accumulating drusen may activate the CS, trigger the local production of inflammatory mediators, and attract leukocytes that in turn augment the local inflammatory state present in AMD.

Current treatment options for AMD include photodynamic therapy with benzoporphyrin (Arch Ophthalmol (1999) 117: 1329-1345) and a number of therapies which target the Vascular Endothelial Growth Factor (VEGF) pathway. Examples of such VEGF-targeted therapies include antibodies such as ranibizumab (marketed as Lucentis™, Genentech, Inc.) and
5 bevacizumab (Avastin™, Genentech, Inc.) and aflibercept (Eylea™, Bayer). However, although these anti-VEGF antibody therapies have been very effective, they are only approved for treatment of the wet or neovascular form of AMD, which accounts for approximately 10-15% of all AMD patients. Antibody therapies are administered by monthly intravitreal injection, in an operating theatre or clean-room, which places a burden on
10 patients, who are typically elderly.

There are currently no approved treatments for the early stage or advanced (dry) forms of AMD.

The complement system is a well-documented target for the treatment of many inflammatory diseases. Overactive or improperly-functioning complement system has been implicated in
15 the pathology of many chronic inflammatory conditions, including AMD (Nature Reviews (2015) 14: 857-877). As a consequence, several complement-targeted therapeutics have been proposed, or are currently in development, which target the alternative pathway amplification loop/C3b feedback cycle as a means of decreasing C3b feedback or increasing C3b breakdown (Figure 1).

20 Lampalizumab (Genentech/Roche) is a humanised monoclonal antibody that inhibits Complement Factor D, administered by monthly intravitreal injection to stop the rate of progression of geographic atrophy. Lampalizumab showed some reduction in the rate of geographic atrophy enlargement in a Phase 2 clinical trial. However, in Phase III randomised clinical trials involving 906 participants, lampalizumab failed to reduce GA enlargement when
25 compared with sham over 48 weeks. Results showed substantial and consistent enlargement of GA, at a mean of approximately 2 mm² per year.

However, there remains a need for alternative therapies for the treatment of complement mediated or complement associated diseases, in particular ocular diseases, such as AMD, in particular treatments that are effective in broad AMD populations and not limited to
30 particular genotypes that may predispose an individual to AMD and other complement-related disorders.

SUMMARY OF THE INVENTION

The applicant has identified therapeutic combinations of Complement Factor I (CFI) and CFI cofactors (proteins that have cofactor activity in the CFI-mediated cleavage of C3b, such as Complement Factor H Like Protein 1 (FHL1), Complement Factor H (CFH), Complement Receptor type 1 (CR1) and Membrane Cofactor Protein (MCP)) for delivery to a patient for restoration or rebalancing of overactive complement C3b feedback cycle. The combinations provide CFI and CFI cofactor at molar ratios that ensure the cofactor is provided in stoichiometric excess to CFI to ensure maximum activity of CFI in C3b breakdown (and downregulation of overactive complement system). This is important for patients who may have genetic deficiencies in certain complement system proteins and/or for treatments that are administered to tissue or organs in which co-factor levels may be reduced in comparison to systemic levels, for example, in the eye or kidney.

In addition, the applicant has provided bicistronic vectors that can be used for the delivery and co-expression of both CFI and CFI cofactor (e.g. FHL1) to a patient. In particular, the applicant has successfully designed functional AAV vectors that can be produced at good titres and comprise nucleotide sequences encoding both CFI and cofactor, thus overcoming challenges posed by the limited capacity of AAV. The bicistronic vectors of the invention also advantageously enable good expression of both CFI and cofactor; and co-expression of CFI and cofactor at ratios that have been identified herein as beneficial.

In one aspect, the invention provides a product comprising (i) a Complement Factor I (CFI) cofactor; and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor, as a combined preparation for simultaneous, separate or sequential use in therapy.

In another aspect, the invention provides a product comprising (i) Complement Factor H Like Protein 1 (FHL1) or Complement Factor H (CFH); and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor, as a combined preparation for simultaneous, separate or sequential use in therapy.

In particular embodiments, the product is used in the treatment of complement-mediated disorders, particularly chronic inflammatory conditions and even more particularly, those which are associated with overactivity of the complement C3b feedback cycle.

In another aspect, the invention provides a product comprising (i) a Complement Factor I (CFI) cofactor; and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor, as a combined preparation for simultaneous, separate or sequential use in treating or preventing a complement-mediated disorder, preferably a complement-mediated disorder of the eye.

In another aspect, the invention provides a product comprising (i) Complement Factor H Like Protein 1 (FHL1) or Complement Factor H (CFH); and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor, as a combined preparation for simultaneous, separate or sequential use in treating or preventing a complement-mediated disorder of the eye.

In some embodiments, the Complement Factor I (CFI) cofactor is selected from the group consisting of Complement Factor H Like Protein 1 (FHL1); Complement Factor H (CFH); Complement Receptor 1 (CR1) or a fragment thereof; and Membrane Cofactor Protein (MCP) or a fragment thereof.

10 In some embodiments, the Complement Factor I (CFI) cofactor is Complement Factor H (CFH). In some embodiments, the Complement Factor I (CFI) cofactor is Complement Receptor 1 (CR1) or a fragment thereof. In some embodiments, the Complement Factor I (CFI) cofactor is Membrane Cofactor Protein (MCP) or a fragment thereof.

In preferred embodiments, the Complement Factor I (CFI) cofactor is Complement Factor H Like Protein 1 (FHL1).

In preferred embodiments, the product comprises (i) Complement Factor H Like Protein 1 (FHL1); and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor. In other embodiments, the product comprises (i) Complement Factor H (CFH); and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor. In other

20 other embodiments, the product comprises (i) Complement Receptor 1 (CR1) or a fragment thereof; and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor. In other embodiments, the product comprises (i) Membrane Cofactor Protein (MCP) or a fragment thereof; and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor.

25 In some embodiments, the disorder is associated with over-activity of the complement C3b feedback cycle and/or under-activity of the C3b breakdown cycle (see Figure 1).

In some embodiments, the disorder is a chronic complement-mediated inflammatory condition of the eye.

In some embodiments, the disorder is age-related macular degeneration (AMD) or diabetic retinopathy. In other embodiments, the disorder is glaucoma, Stargardt's disease, central serous chorioretinopathy or retinitis pigmentosa.

In preferred embodiments, the disorder is AMD. In some embodiments, the AMD is dry AMD.

In some embodiments, the product provides (i) and (ii) to a subject in a (i):(ii) molar ratio of at least 2:1, at least 3:1, at least 4:1, at least 5:1, at least 6:1, at least 7:1, at least 8:1, at least 9:1, at least 10:1, at least 15:1, at least 20:1, at least 25:1, at least 30:1, at least 40:1, at least 50:1 or at least 60:1.

In some embodiments, the product provides (i) and (ii) to a subject in a (i):(ii) molar ratio of at least 2:1. In some embodiments, the product provides (i) and (ii) to a subject in a (i):(ii) molar ratio of at least 3:1. In preferred embodiments, the product provides (i) and (ii) to a subject in a (i):(ii) molar ratio of at least 8:1.

In some embodiments, the product provides (i) and (ii) to a subject in a (i):(ii) molar ratio of between 2:1 and 34:1, between 2:1 and 25:1, between 2:1 and 15:1, between 2:1 and 12:1, between 3:1 and 10:1.

In preferred embodiments, the product provides (i) and (ii) to a subject in a (i):(ii) molar ratio of between 3:1 and 10:1.

The provided molar ratios may be achieved through, for example, delivery of protein, polynucleotide or vector to a subject. Protein levels may be readily measured by the skilled person using techniques known in the art, such as ELISA, for example as described herein. Likewise, amounts of proteins expressed from polynucleotides or vectors encoding therefor may be measured using similar approaches.

In another aspect, the invention provides an isolated polynucleotide comprising nucleotide sequences encoding (i) a Complement Factor I (CFI) cofactor; and (ii) Complement Factor I (CFI).

In another aspect, the invention provides an isolated polynucleotide comprising nucleotide sequences encoding (i) Complement Factor H Like Protein 1 (FHL1) or Complement Factor H (CFH); and (ii) Complement Factor I (CFI).

In some embodiments, the Complement Factor I (CFI) cofactor is selected from the group consisting of Complement Factor H Like Protein 1 (FHL1); Complement Factor H (CFH); Complement Receptor 1 (CR1) or a fragment thereof; and Membrane Cofactor Protein (MCP) or a fragment thereof.

In some embodiments, the Complement Factor I (CFI) cofactor is Complement Factor H (CFH). In some embodiments, the Complement Factor I (CFI) cofactor is Complement Receptor 1 (CR1) or a fragment thereof. In some embodiments, the Complement Factor I (CFI) cofactor is Membrane Cofactor Protein (MCP) or a fragment thereof.

- 5 In preferred embodiments, the Complement Factor I (CFI) cofactor is Complement Factor H Like Protein 1 (FHL1).

In preferred embodiments, the polynucleotide comprises nucleotide sequences encoding (i) Complement Factor H Like Protein 1 (FHL1); and (ii) Complement Factor I (CFI). In other
10 embodiments, the polynucleotide comprises nucleotide sequences encoding (i) Complement Factor H (CFH); and (ii) Complement Factor I (CFI). In other embodiments, the polynucleotide comprises nucleotide sequences encoding (i) Complement Receptor 1 (CR1) or a fragment thereof; and (ii) Complement Factor I (CFI). In other embodiments, the polynucleotide comprises nucleotide sequences encoding (i) Membrane Cofactor Protein (MCP) or a fragment thereof; and (ii) Complement Factor I (CFI).

- 15 In some embodiments, the polynucleotide further comprises a nucleotide sequence encoding a CMV promoter. Preferably, the CMV promoter is upstream of the nucleotide sequences encoding the (i) and (ii).

In some embodiments, the polynucleotide further comprises a nucleotide sequence encoding a WPRE regulatory element. Preferably, the WPRE regulatory element is
20 downstream of the nucleotide sequences encoding the (i) and (ii).

In some embodiments, the polynucleotide further comprises a nucleotide sequence encoding a poly-A signal. Preferably, wherein the poly-A signal is downstream of the nucleotide sequences encoding the (i) and (ii).

- 25 In some embodiments, the polynucleotide further comprises a nucleotide sequence encoding a Bovine Growth Hormone poly-A signal. Preferably, wherein the Bovine Growth Hormone poly-A signal is downstream of the nucleotide sequences encoding the (i) and (ii).

In some embodiments, the polynucleotide further comprises nucleotide sequences encoding:

- (a) a CMV promoter, wherein the CMV promoter is upstream of the nucleotide sequences encoding the (i) and (ii); and

- (b) a Bovine Growth Hormone poly-A signal, wherein the Bovine Growth Hormone poly-A signal is downstream of the nucleotide sequences encoding the (i) and (ii).

In other embodiments, the polynucleotide further comprises nucleotide sequences encoding:

- 5 (a) a CMV promoter, wherein the CMV promoter is upstream of the nucleotide sequences encoding the (i) and (ii);
- (b) a WPRE regulatory element, wherein the WPRE regulatory element is downstream of the nucleotide sequences encoding the (i) and (ii); and
- 10 (c) a Bovine Growth Hormone poly-A signal, wherein the Bovine Growth Hormone poly-A signal is downstream of the nucleotide sequences encoding the (i) and (ii).

In preferred embodiments, the WPRE regulatory element is a WPRE3 regulatory element.

In preferred embodiments, the nucleotide sequence encoding (i) is upstream of the nucleotide sequence encoding (ii).

- 15 In other embodiments, the nucleotide sequence encoding (ii) is upstream of the nucleotide sequence encoding (i).

- In some embodiments, the nucleotide sequences encoding the (i) and (ii) are operably linked by a linker. In some embodiments, the linker is a Furin, GSG, 11aa1D or F2A linker. In preferred embodiments, the linker contains a self-cleaving 2A peptide sequence, for example P2A or a sequence which comprises or is defined by a Furin cleavage site, GSG, 11a1D and an F2A sequence.
- 20

In some embodiments, the polynucleotide further comprises one or more adeno-associated virus (AAV) inverted terminal repeats (ITRs). In preferred embodiments, the polynucleotide further comprises two AAV ITRs.

- 25 In some embodiments, the polynucleotide comprises an AAV ITR at its 5' end and an AAV ITR at its 3' end.

In some embodiments, the polynucleotide comprises:

- (a) a 5' AAV ITR;
- (b) a CMV promoter;

- (c) a nucleotide sequence encoding a Complement Factor I (CFI) cofactor, preferably FHL1;
- (d) a linker, optionally wherein the linker comprises a Furin cleavage site, GSG, 11a1D and an F2A sequence;
- 5 (e) a nucleotide sequence encoding CFI;
- (f) a poly-A signal, preferably a Bovine Growth Hormone poly-A signal; and
- (g) a 3' AAV ITR.

In some embodiments, the polynucleotide comprises:

- (a) a 5' AAV ITR;
- 10 (b) a CMV promoter;
- (c) a nucleotide sequence encoding a Complement Factor I (CFI) cofactor, preferably FHL1;
- (d) a linker, optionally wherein the linker comprises a Furin cleavage site, GSG, 11a1D and an F2A sequence;
- 15 (e) a nucleotide sequence encoding CFI;
- (f) a WPRE regulatory element, preferably wherein the WPRE regulatory element is a WPRE3 regulatory element;
- (g) a poly-A signal, preferably a Bovine Growth Hormone poly-A signal; and
- (h) a 3' AAV ITR.

20 In preferred embodiments, the polynucleotide comprises:

- (a) a 5' AAV ITR;
- (b) a CMV promoter;
- (c) a nucleotide sequence encoding FHL1;
- (d) a linker comprising a Furin cleavage site, GSG, 11a1D and an F2A sequence;
- 25 (e) a nucleotide sequence encoding CFI;

- (f) a WPRE3 regulatory element;
- (g) a poly-A signal, preferably a Bovine Growth Hormone poly-A signal; and
- (h) a 3' AAV ITR.

5 In some embodiments, the Complement Factor I (CFI) cofactor is selected from the group consisting of Complement Factor H Like Protein 1 (FHL1); Complement Factor H (CFH); Complement Receptor 1 (CR1) or a fragment thereof; and Membrane Cofactor Protein (MCP) or a fragment thereof.

10 In some embodiments, the Complement Factor I (CFI) cofactor is Complement Factor H (CFH). In some embodiments, the Complement Factor I (CFI) cofactor is Complement Receptor 1 (CR1) or a fragment thereof. In some embodiments, the Complement Factor I (CFI) cofactor is Membrane Cofactor Protein (MCP) or a fragment thereof.

In preferred embodiments, the Complement Factor I (CFI) cofactor is Complement Factor H Like Protein 1 (FHL1).

15 In some embodiments, the AAV ITRs are AAV2 or AAV8 ITRs. In preferred embodiments, the AAV ITRs are AAV2 ITRs.

20 In some embodiments, the nucleotide sequences encoding the Complement Factor I (CFI) cofactor, such as FHL1 or CFH, are codon optimised. In some embodiments, the nucleotide sequence encoding CFI is codon optimised. In preferred embodiments, the nucleotide sequences encoding the Complement Factor I (CFI) cofactor, such as FHL1 or CFH, and CFI are codon optimised.

In some embodiments, the nucleotide sequence encoding FHL1 has at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 12.

In preferred embodiments, the nucleotide sequence encoding FHL1 is SEQ ID NO: 12.

25 In some embodiments, the nucleotide sequence encoding CFI has at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 10.

In preferred embodiments, the nucleotide sequence encoding CFI is SEQ ID NO: 10.

30 In preferred embodiments, the nucleotide sequence encoding FHL1 is SEQ ID NO: 12 and the nucleotide sequence encoding CFI is SEQ ID NO: 10.

In some embodiments, the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 22, or a nucleotide sequence that has at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereto.

5 In some embodiments, the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 23, or a nucleotide sequence that has at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereto.

In some embodiments, the polynucleotide is less than or equal to 5.2, 5.1, 5.0, 4.9, 4.8 or 4.7 kb. In preferred embodiments, the polynucleotide is less than or equal to 4.7 kb.

10 In another aspect, the invention provides a vector comprising the polynucleotide of the invention.

In some embodiments, the vector is an adeno-associated viral (AAV), retroviral, lentiviral or adenoviral vector.

In preferred embodiments, the vector is an AAV vector.

In some embodiments, the vector is in the form of a viral vector particle.

15 In some embodiments, the AAV vector particle comprises an AAV2 or AAV8 genome.

In some embodiments, the AAV vector particle comprises AAV2 or AAV8 capsid proteins.

20 In some embodiments, the AAV vector particle comprises an AAV2 genome and AAV2 capsid proteins (AAV2/2). In other embodiments, the AAV vector particle comprises an AAV2 genome and AAV8 capsid proteins (AAV2/8). In other embodiments, the AAV vector particle comprises an AAV8 genome and AAV8 capsid proteins (AAV8/8).

In another aspect, the invention provides a cell comprising the polynucleotide of the invention.

In another aspect, the invention provides a cell transduced with the vector of the invention.

25 In another aspect, the invention provides a pharmaceutical composition comprising the polynucleotide, vector or cell of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

In preferred embodiments, the pharmaceutical composition is for intraocular administration.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in therapy.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in treating or preventing an ocular disorder.

- 5 In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in treating or preventing a complement-mediated or a complement-associated disorder.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in treating or preventing a complement-mediated disorder of the eye.

- 10 In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in treating or preventing a complement-mediated or complement-associated renal disorder or complement-mediated or complement-associated disorder of the central nervous system (CNS).

- In another aspect, the invention provides a method of treating or preventing a complement-mediated or complement associated disorder of the eye comprising administering the polynucleotide, vector or cell of the invention to a subject in need thereof.
- 15

- In another aspect, the invention provides a method of providing (i) a Complement Factor I (CFI) cofactor; and (ii) Complement Factor I (CFI) to a subject, comprising delivering the polynucleotide, vector or cell of the invention to the subject, preferably to the eye of the subject.
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In another aspect, the invention provides a method of providing (i) Complement Factor H Like Protein 1 (FHL1) or Complement Factor H (CFH); and (ii) Complement Factor I (CFI) to a subject, comprising delivering the polynucleotide, vector or cell of the invention to the eye of the subject.

- 25 In some embodiments, the disorder is associated with over-activity of the complement C3b feedback cycle and/or under-activity of the C3b breakdown cycle (see Figure 1).

In some embodiments, the disorder is a chronic complement-mediated or chronic complement-associated inflammatory condition.

- In some embodiments, the disorder is a chronic complement-mediated inflammatory condition of the eye.
- 30

In some embodiments, the disorder is age-related macular degeneration (AMD) or diabetic retinopathy. In other embodiments, the disorder is glaucoma, Stargardt's disease, central serous chorioretinopathy, retinitis pigmentosa or uveitis. Preferably, the uveitis is posterior uveitis.

- 5 In preferred embodiments, the disorder is AMD. In some embodiments, the AMD is dry AMD.

In some embodiments, a subject has been diagnosed with AMD or is at risk of acquiring AMD.

In some embodiments, the use is for treating or preventing a disorder in a subject:

- 10 (a) having lower than normal Complement Factor I activity or concentration in the eye and/or serum, preferably having a concentration of, or activity equivalent to, 0-30, 0-20 or 0-10 $\mu\text{g/mL}$ in serum; and/or
- (b) being heterozygous or homozygous for an age-related macular degeneration (AMD)-associated SNP, preferably a rare Complement
- 15 Factor I variant.

In some embodiments, the use is for treating or preventing a disorder in a subject:

- (a) having a normal level of Complement Factor I activity or concentration in the eye and/or serum, preferably at least 30 $\mu\text{g/mL}$, such as 30-40 $\mu\text{g/mL}$ in serum; and/or
- 20 (b) not carrying a rare Complement Factor I variant allele.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in treating or preventing age-related macular degeneration (AMD). In preferred embodiments, the AMD is dry AMD.

- 25 In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in treating or preventing diabetic retinopathy.

In some embodiments, the formation of geographic atrophy is prevented or reduced, and/or the amount of geographic atrophy is reduced.

In some embodiments, the progression of geographic atrophy is slowed.

In some embodiments, there is at least a 10% reduction in the increase in geographic atrophy area over the 12 months following administration to a treated eye of a subject, relative to an untreated eye over the same period. In other embodiments, there is at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% reduction in the increase in geographic atrophy area over the 12 months following administration to a treated eye of a subject, relative to an untreated eye over the same period

In some embodiments, administration of the polynucleotide, vector or cell increases the level of C3b-inactivating and iC3b-degradation activity in a subject, or in an eye, such as in the retinal pigment epithelium (RPE), of a subject, optionally to a level that exceeds a normal level in a subject, or eye or RPE thereof.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in improving or restoring vision or visual acuity, for example in a subject suffering from an eye disorder, such as an eye disorder disclosed herein. In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in mitigating loss of vision or visual acuity, for example a loss of vision or visual acuity associated with an eye disorder, such as an eye disorder disclosed herein.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in improving or restoring reading speed in a subject, for example in a subject suffering from an eye disorder, such as an eye disorder disclosed herein. In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in mitigating reduction in reading speed in a subject, for example a reduction in reading speed associated with an eye disorder, such as an eye disorder disclosed herein.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in reducing or preventing loss of photoreceptors and/or the retinal pigment epithelium (RPE), for example a loss of photoreceptors and/or the RPE associated with an eye disorder, such as an eye disorder disclosed herein.

In some embodiments, the polynucleotide, vector or cell is administered intraocularly.

In some embodiments, the polynucleotide, vector or cell is administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection.

In some embodiments, the polynucleotide, vector or cell is administered to the eye of a subject by subretinal injection.

In some embodiments, the polynucleotide or vector of the invention does not comprise a hAAT promoter. In some embodiments, the polynucleotide or vector of the invention does not comprise an ApoR enhancer. In other embodiments, the polynucleotide or vector of the invention does not comprise two ApoR enhancers.

- 5 In some embodiments, the vector of the invention does not comprise an AAV2 genome and an AAV8 capsid protein, i.e. the vector of the invention is not an AAV2/8 vector.

In some embodiments, the polynucleotide, vector or cell of the invention is not administered systemically. In other embodiments, the polynucleotide, vector or cell of the invention is not administered intravenously.

- 10 It will be understood by those skilled in the art that the descriptions above relating to the treatment of AMD are based on a theory of modulating hyperactivated complement system (either as a result of overactive C3b feedback cycle and/or underactive C3b breakdown cycle) and therefore (with the exception of text specifically relating to intraocular administration) the description applies equally to other chronic inflammatory conditions in
15 which the complement system is implicated. Such disorders may be treated by administration of the products, proteins, vectors, cells and compositions described herein by systemic administration (for example via peripheral vein infusion), local administration (for example intrathecally) or direct delivery to targeted tissue or organs (for example, liver, kidney).

20 DESCRIPTION OF THE DRAWINGS

FIGURE 1

C3b feedback (amplification) and breakdown (down-regulation) cycles of the alternative pathway of vertebrate complement ("I" = Complement Factor I; "H" = Complement Factor H; "B" = Complement Factor B; and "D" = Complement Factor D).

25 FIGURE 2

(A) Cofactor assay based on ELISA-based measurement of cleavage of C3b to iC3b. Concentrations of CFI and C3b were fixed, and titrations of CFH or FHL1 in specified ratios were carried out.

- (B) LPS deposition assay based on supplementation of normal serum with Complement
30 Factor I (FI), Complement Factor H (FH) or Complement Factor H-like Protein 1 (FHL1).

FIGURE 3

Western blot analyses of supernatants from vector transduction of HEK293 cells (RC001, control vector comprising wild-type FHL1; GT005, control vector comprising wild-type CFI).

FIGURE 4

ELISA analyses of supernatants from vector transduction of HEK293 cells (RC001, control vector comprising wild-type FHL1; GT005, control vector comprising wild-type CFI).

FIGURE 5

Alkaline gel analyses of vector genome packaging (top panel). Comparison of the ratio of full:empty viral particles determined by qPCR and capsid ELISA (bottom panel). (RC001, control vector comprising wild-type FHL1; GT005, control vector comprising wild-type CFI).

FIGURE 6

C3b cleavage assays using Western blot analysis (top panel) and ELISA (bottom panel). (RC001, control vector comprising wild-type FHL1; GT005, control vector comprising wild-type CFI).

FIGURE 7

Comparison of Complement Factor I (FI):Complement Factor H (FH) ratios in blood plasma (n=80) and vitreous humour (n=29).

FIGURE 8

LPS deposition assay to measure C3 deposition. FI = Complement Factor I, sCR1 = soluble complement receptor 1, FH = Complement Factor H, FHL1 = Factor H-like protein 1. Number above each bar shows the % of reduction in C3 deposition compared to serum only.

FIGURE 9

Western blot analysis of supernatant of in vitro transduced HEK-293 cells. UTC = untransduced cells, GT005 = AAV expressing CFI, GT007 = AAV expressing CFI and FHL1, RC001 = AAV expressing FHL1.

FIGURE 10

C3b Western blot and iC3b ELISA of cofactor assay to test functional activity of constructs. (A) Western blot of C3b cofactor assay. (B) iC3b ELISA of C3b cofactor assay. C3b =

Complement C3b, CFI = Complement Factor I, FHL1 = Complement Factor H-like protein 1, UTD = untransduced cells.

FIGURE 11

Isolectin-stained area in choroidal flatmounts. Isolectin stained area data were non-normally distributed as assessed by Kolmogorov-Smirnov test and statistical significance of the observed differences was determined using Generalised Linear Model analysis. * = $P < 0.05$; *** = $P < 0.0001$, **** = $P < 0.00001$. Dots represent results from individual laser burns, the line represents the mean value.

DETAILED DESCRIPTION OF THE INVENTION

The terms “comprising”, “comprises” and “comprised of” as used herein are synonymous with “including” or “includes”; or “containing” or “contains”, and are inclusive or open-ended and do not exclude additional, non-recited members, elements or steps. The terms “comprising”, “comprises” and “comprised of” also include the term “consisting of”.

COMPLEMENT SYSTEM

The complement system is an integral part of the humoral immune system and is involved in tissue inflammation, cell opsonisation, and cytolysis. It provides protection against microorganisms and mediates the clearance of exogenous and endogenous cellular debris from the host tissues.

The complement system cascade is comprised of three activation pathways. All of the pathways ultimately end in the central cleavage of C3 factor and in the generation of its active fragments C3a and C3b. C3a is the anaphylatoxin that triggers a range of chemotactic and proinflammatory responses, such as recruitment of inflammatory cells and increased microvasculature permeability, whereas C3b is responsible for opsonisation of foreign surfaces covalently attached to C3b. Opsonisation with activated C3 fragments (C3b and iC3b) fulfils three major functions: (i) cell debris elimination by phagocytic cells (e.g. macrophages or microglia) and the stimulation of the adaptive immune system (B and T cells); (ii) amplification of complement activation via the formation of a surface-bound C3 convertase; and (iii) assemblage of the C5 convertase.

Assemblage of the C5 convertase is responsible for C5 cleavage, which results in the formation of the cytolytic membrane attack complex (MAC) capable of generating perforations in the cell membrane, thereby promoting cell lysis and the elimination of unnecessary cells. Through all of these activities, the innate complement cascade supports

and promotes the function of downstream mechanisms of the immune system that protect the integrity of the host tissue. Overall, complement system pathway activation results in a proinflammatory response, including MAC generation, which mediates cell lysis, the release of chemokines to attract inflammatory cells to the site of damage, and the enhancement of capillary permeability to promote extravasation of infiltrating leukocytes. Under physiological conditions, complement activation is effectively controlled by the coordinated action of soluble and membrane-associated complement regulatory molecules (CRMs). Soluble complement regulators, such as C1-inhibitor, anaphylatoxins inhibitor, C4b binding protein (C4BP), Complement Factor H (CFH), Complement Factor I (CFI), clusterin and vitronectin, restrict the action of complement in human tissues at multiple sites of the cascade reaction. In addition, each individual cell is protected against the attack of homologous complement by surface proteins, such as the Complement Receptor 1 (CR1, CD35), the membrane cofactor protein (CD46), and glycosylphosphatidylinositol-anchored proteins, such as decay-accelerating factor (CD55) or CD59 molecule. Of note, host cells and tissues that are inadequately protected from complement attack might be subjected to bystander cell lysis.

The invention relates to the treatment or prevention of a complement-mediated disorder, for example of the eye. For example, the complement-mediated disorder may be a disorder associated with a defect in alternative pathway regulation, and in particular with over-activity of the complement C3b feedback cycle and/or under-activity of the C3b breakdown cycle.

In some embodiments, prior to administration of the product, polynucleotide, vector, cell or pharmaceutical composition of the invention, the subject has low levels (e.g. lower than normal levels) of Complement Factor I activity, for example low levels of Complement Factor I activity in the eye and/or low serum levels of Complement Factor I activity. The sub-normal level of Complement Factor I activity may be due to sub-normal expression of normally-functioning Complement Factor I, or at least partial (e.g. heterozygous) expression (at normal or sub-normal levels) of a non- or sub-functional variant of Complement Factor I. (Such a subject may carry one or more copies of an AMD-associated SNP, for example the subject may be homo- or heterozygous for one of the rare Complement Factor I variants discussed further below). Thus, the subject may have a low concentration (e.g. a lower than normal concentration) of Complement Factor I in the eye and/or serum. For a human subject, the normal level of Complement Factor I activity (C3b-inactivating and iC3b-degradation activity) may be equivalent to that provided by 30-40 µg/mL Complement Factor I in the serum of the subject. Thus, in a subject with low Complement Factor I activity, the Complement Factor I activity in the serum may correspond to less than 30 µg/mL and greater than 0 µg/mL Complement Factor I, such as 0-20 or 0-10 µg/mL (these being ranges

of Complement Factor I serum concentration which may encompass a subject having a low Complement Factor I concentration).

Thus, the subject to be treated by the invention may suffer from a complement-mediated disorder of the eye such as AMD, more particularly dry AMD (e.g. characterised by geographic atrophy), or may be at risk of developing such a disorder. For example, the subject may be homozygous or heterozygous susceptible for one or more SNPs associated with the complement-mediated disorder.

In some embodiments, the subject is at risk of developing AMD. For example, the subject may be homozygous or heterozygous susceptible for one or more SNPs associated with AMD, for example rare mutations in Complement Factor I associated with advanced AMD which commonly result in reduced serum Complement Factor I levels (Kavanagh et al. (2015) Hum Mol Genet 24: 3861-3870). In particular the subject may carry one or two copies of one or more of the following rare Complement Factor I variants: rs144082872 (encoding P50A); 4:110687847 (encoding P64L); rs141853578 (encoding G119R); 4:110685721 (encoding V152M); 4:110682846 (encoding G162D); 4:110682801 (encoding N177I); rs146444258 (encoding A240G); rs182078921 (encoding G287R); rs41278047 (encoding K441R); and rs121964913 (encoding R474).

The invention may further comprise determining whether the subject is at risk of developing a complement-mediated disorder (for example, AMD), for example by determining whether the subject is homozygous or heterozygous susceptible for one or more SNPs associated with the complement-mediated disorder (for example, by determining whether the subject is homozygous or heterozygous susceptible for one or more of the rare Complement Factor I variants associated with AMD listed above).

Alternatively, the subject may have a normal level of endogenous Complement Factor I activity or concentration, for example in the eye and/or serum and/or may not carry a rare variant Complement Factor I allele.

In some embodiments, administration of the product, polynucleotide, vector, cell or pharmaceutical composition of the invention thereby increases the level of C3b-inactivating and iC3b-degradation activity in the eye of the subject. In other embodiments, administration of the product, polynucleotide, vector, cell or pharmaceutical composition of the invention thereby increases the level of C3b-inactivating and iC3b-degradation activity in the eye of the subject to a level that exceeds a normal level in the eye. More particularly, the level of C3b-inactivating and iC3b-degradation activity is increased in the RPE of the eye.

It will be appreciated that the C3b-inactivating and iC3b-degradation activity in the subject following provision of the product of the invention and/or expression of the Complement Factor I and CFI cofactor, such as Complement Factor H-like Protein 1, from the polynucleotide or vector of the invention may comprise C3b-inactivating and iC3b-degradation activity from the subject's endogenous Complement Factor I (i.e. the subject's Complement Factor I not provided by the product or produced by expression from the polynucleotide or vector), and C3b-inactivating and iC3b-degradation activity provided by the product of the invention or produced by expression from the polynucleotide or vector of the invention, such that the total level of C3b-inactivating and iC3b-degradation activity in the subject exceeds a normal level.

In some embodiments, the level of C3b-inactivating and iC3b-degradation activity in the subject, for example in the eye, is increased to a level that is at least 5%, 10%, 15%, 20% or 25% above the normal level.

In other embodiments, the level of C3b-inactivating and iC3b-degradation activity in the subject, for example in the eye, is increased to a level that is up to twice the normal level, or up to 80%, 60%, 40% or 20% above the normal level.

For example, the level of C3b-inactivating and iC3b-degradation activity in the subject, for example in the eye, may be increased to a level that is 5-100%, 5-80%, 5-60%, 5-40%, 5-20%, 10-100%, 10-80%, 10-60%, 10-40%, 10-20%, 15-100%, 15-80%, 15-60%, 15-40%, 15-20%, 20-100%, 20-80%, 20-60%, 20-40%, 25-100%, 25-80%, 25-60% or 25-40% above the normal level.

In some embodiments, administration of the product, polynucleotide, vector, cell or pharmaceutical composition of the invention does not detectably increase the level of C3b-inactivating and iC3b-degradation activity in the plasma/serum of the subject. In other embodiments, administration of the product, polynucleotide, vector, cell or pharmaceutical composition of the invention does not detectably increase the level of C3b-inactivating and iC3b-degradation activity in the plasma/serum of the subject to a level greater than the normal level.

In the foregoing section, except where obviously inapplicable, reference to Complement Factor I and C3b-inactivating and iC3b-degradation activity may be replaced with a CFI cofactor, preferably Complement Factor H or Complement Factor H-like Protein 1, and ability to act as a cofactor for the Complement Factor I mediated cleavage of C3b and to increase the rate of dissociation of C3 convertase and C5 convertase, respectively. In some embodiments, prior to administration of the product, polynucleotide, vector, cell or

pharmaceutical composition of the invention, the subject has low levels (e.g. lower than normal levels) of Complement Factor H, for example low levels of Complement Factor H in the eye and/or low serum levels of Complement Factor H. For a human subject, the normal level of Complement Factor H may be about 200-500 µg/mL in the serum of the subject.

- 5 Thus, in a subject with low levels of Complement Factor H, the levels in the serum may be less than 200 µg/mL and greater than 0 µg/mL, such as 0-100 µg/mL. Alternatively, the subject may have a normal level of endogenous Complement Factor H, for example in the eye and/or serum.

COMPLEMENT FACTOR I (CFI)

- 10 Complement Factor I (Factor I, CFI), also known as C3b/C4b inactivator, is a protein that in humans is encoded by the *CFI* gene.

- Complement Factor I is a serine protease that circulates in a zymogen-like state (Roversi et al. (2011) PNAS 108: 12839-12844) at a concentration of ~35 µg/mL (Nilsson et al. (2011) Mol Immunol 48: 1611-1620). The Complement Factor I protein is a heavily N-glycosylated
15 heterodimer consisting of two polypeptide chains linked by a single disulfide bond. The heavy chain (50 kDa) comprises an N-terminal region; an FI membrane attack complex (FIMAC) domain; a CD5 like-domain or scavenger receptor cysteine-rich (SRCR) domain; two low-density lipoprotein receptor (LDLr) domains; and a C-terminal region of unknown function that is a site of sequence variability across species (Roversi et al. (2011) PNAS 108:
20 12839-12844). The light chain (38 kDa) contains the serine protease (SP) domain with the conserved catalytic residues (Goldberger et al. (1987) J Biol Chem 262: 10065-10071).

- Complement Factor I inactivates C3b by cleaving it into iC3b, C3d and C3d,g and, in an analogous way, C4b into C4c and C4d. To properly perform its functions, Complement Factor I requires the presence of cofactor proteins such as C4b-Binding Protein (C4BP),
25 Complement Factor H (CFH), Complement Receptor 1 (CR1/CD35) and Membrane Cofactor Protein (MCP/CD46) (Degn et al. (2011) Am J Hum Genet 88: 689-705).

- iC3b is incapable of associating with Factor B, and thus cannot perpetuate amplification of the complement cascade or activation through the alternative pathway. Hence, once C3b has been cleaved to iC3b, neither alternative pathway initiation nor terminal complement
30 cascade activation occurs.

iC3b is capable of providing a proinflammatory action by binding to, and activating, Complement Receptor 3 (CR3)(CD11b/CD18) on polymorphonuclear leukocytes (mostly neutrophils), NK cells and mononuclear phagocytes, such as macrophages.

Complement Factor I is capable of processing iC3b into C3dg via a protease activity requiring the cofactor, CR1. C3dg is unable to bind to CR3. Since iC3b reacting with the complement receptor CR3 is a major mechanism by which complement activation gives rise to inflammation, the breakdown of iC3b to C3dg is essential for reducing complement-induced inflammation (Lachmann (2009) Adv. Immunol. 104: 115-149).

Complement Factor I's unique ability to both promote cleavage of C3b to iC3b as well as accelerate breakdown of iC3b – combined with its relatively low concentration in human serum, with implications for the amount required to be delivered for therapeutic efficacy – make it a particularly advantageous target.

10 In some embodiments, a Complement Factor I polypeptide is capable of cleaving C3b into an inactive degradation product. For example, the Complement Factor I polypeptide may be capable of cleaving C3b into iC3b.

In some embodiments, a Complement Factor I polypeptide is capable of processing iC3b into an inactive degradation product. For example, the Complement Factor I polypeptide
15 may be capable of processing iC3b into C3dg.

In preferred embodiments, the Complement Factor I polypeptide is capable of cleaving C3b into iC3b and processing iC3b into C3dg.

Suitably, a fragment or derivative of Complement Factor I may retain at least 50%, 60%, 70%, 80%, 90%, 95% or 100% of the C3b-inactivating and iC3b-degradation activity of
20 native Complement Factor I.

The C3b-inactivating and iC3b-degradation activity of Complement Factor I, or a fragment or derivative thereof, may be determined using any suitable method known to the skilled person. For example, measurement of Complement Factor I proteolytic activity is described in Hsiung et al. (Biochem. J. (1982) 203: 293-298). Both haemolytic and conglutinating
25 assays for CFI activity are described in Lachmann PJ & Hobart MJ (1978) "Complement Technology" in Handbook of Experimental Immunology 3rd edition Ed DM Weir Blackwells Scientific Publications Chapter 5A p17. A more detailed description, also including a proteolytic assay, is given by Harrison RA (1996) in "Weir's Handbook of Experimental Immunology" 5th Edition Eds; Herzenberg Leonore A'Weir DM, Herzenberg Leonard A &
30 Blackwell C Blackwells Scientific Publications Chapter 75 36-37. The conglutinating assay is highly sensitive and can be used for detecting both the first (double) clip converting fixed C3b to iC3b and acquiring reactivity with conglutinin; and for detecting the final clip to C3dg by starting with fixed iC3b and looking for the loss of reactivity with conglutinin. The

haemolytic assay is used for the conversion of C3b to iC3b, and the proteolytic assay detects all the clips.

In some embodiments, the Complement Factor I is human Complement Factor I.

An example human Complement Factor I protein is the human Complement Factor I protein having the UniProtKB accession number P05156. This exemplified sequence is 583 amino acids in length (disclosed as SEQ ID NO: 1) of which amino acids 1 to 18 form a signal sequence.

In some embodiments, the amino acid sequence of Complement Factor I is SEQ ID NO: 1. In other embodiments, the amino acid sequence of Complement Factor I is the sequence disclosed as positions 19 to 583 of SEQ ID NO: 1.

15 MKLLHVFLLFLCFHLRFCKVTYTSQEDLVEKKCLAKKYTHLSCDKVFCQPWQRCIEGTCVCKLPYQCPKNGT
AVCATNRRSFPTYCQQKSLECLHPGTFKFLNNGTCTAEGKFSVSLKHGNTDSEGIVEVKLVLDQDKTMFICKSS
WSMREANVACLDLGFQQGADTQRRFKLSDLINSTECLHVHCRGLETSLAECTFTKRRTMGYQDFADVVCYT
QKADSPMDFFQCVNGKYISQMKACDGINDCGDSDELCKACQKGKGFHCKSGVCIPSQYQCNGEVDCTGE
DEVGCAGFASVTQEETEILTADMDAERRRIKSLLPKLSCGVKNRMHIRRKRIVGGKRAQLGDLWPQVAIKDA
SGITCGGIYIGGCWILTAACHLRASKTHRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIAL
IEMKKDGNKKDCELPRIPACVPWSPYLFQPNDCIVSGWGREKDNERVFSLQWGEVKLISNCSKFYGNRFY
EKEMECAGTYDGSIDACKGDSGGPLVCMDANNVTYVWGVVSWGENCGKPEFPGVYTKVANYFDWISYHVGRP
FISQYNV

20 (SEQ ID NO: 1)

In some embodiments, the amino acid sequence of Complement Factor I is SEQ ID NO: 9, which corresponds to NCBI Accession No. NP_000195. In other embodiments, the amino acid sequence of Complement Factor I is the sequence disclosed as positions 19 to 583 of SEQ ID NO: 9.

25 MKLLHVFLLFLCFHLRFCKVTYTSQEDLVEKKCLAKKYTHLSCDKVFCQPWQRCIEGTCVCKLPYQCPKNGT
AVCATNRRSFPTYCQQKSLECLHPGTFKFLNNGTCTAEGKFSVSLKHGNTDSEGIVEVKLVLDQDKTMFICKSS
WSMREANVACLDLGFQQGADTQRRFKLSDLINSTECLHVHCRGLETSLAECTFTKRRTMGYQDFADVVCYT
QKADSPMDFFQCVNGKYISQMKACDGINDCGDSDELCKACQKGKGFHCKSGVCIPSQYQCNGEVDCTGE
30 DEVGCAGFASVAQEETEILTADMDAERRRIKSLLPKLSCGVKNRMHIRRKRIVGGKRAQLGDLWPQVAIKDA
SGITCGGIYIGGCWILTAACHLRASKTHRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIAL
IEMKKDGNKKDCELPRIPACVPWSPYLFQPNDCIVSGWGREKDNERVFSLQWGEVKLISNCSKFYGNRFY
EKEMECAGTYDGSIDACKGDSGGPLVCMDANNVTYVWGVVSWGENCGKPEFPGVYTKVANYFDWISYHVGRP
FISQYNV

(SEQ ID NO: 9)

35 An example wild type nucleotide sequence encoding Complement Factor I is the nucleotide sequence having the NCBI Accession No. NM_000204, disclosed herein as SEQ ID NO: 2.

ATGAAGCTTCTTCATGTTTTCTGTATTCTGTGCTTCCACTTAAGGTTTTGCAAGGTCACTTATACATCT
CAAGAGGATCTGGTGGAGAAAAAGTGCTTAGCAAAAAAATATACTCACCTCTCCTGCGATAAAGTCTTCTGC
CAGCCATGGCAGAGATGCATTGAGGGCACCTGTGTTTGTAACTACCGTATCAGTGCCCAAAGAATGGCACT

GCAGTGTGTGCAACTAACAGGAGAAGCTTCCCAACATACTGTCAACAAAAGAGTTTGGAATGTCTTCATCCA
 GGGACAAAGTTTTTAAATAACGGAACATGCACAGCCGAAGGAAAGTTTAGTGTTTCCTTGAAGCATGGAAT
 ACAGATTGAGAGGGAATAGTTGAAGTAAAACTTGTGGACCAAGATAAGACAATGTTTCATATGCAAAAGCAGC
 TGGAGCATGAGGGAAGCCAACGTGGCCTGCCTTGACCTTGGGTTTCAACAAGGTGCTGATACTCAAAGAAGG
 5 TTTAAGTTGTCTGATCTCTCTATAAAATCCACTGAATGTCTACATGTGCATTGCCGAGGATTAGAGACCAGT
 TTGGCTGAATGTACTTTTACTAAGAGAAGAAGTATGGGTTACCAGGATTTGCTGATGTGGTTTGTATACA
 CAGAAAGCAGATTCTCAATGGATGACTTCTTTTCAAGTGTGTGAATGGGAAATACATTTCTCAGATGAAAGCC
 TGTGATGGTATCAATGATTGTGGAGACCAAGTATGAAGTGTGTGTAAGCATGCCAAGGCAAAGGCTTC
 CATTGCAATCGGGTGTTCATTCCAAGCCAGTATCAATGCAATGGTGAGGTGGACTGCATTACAGGGGAA
 10 GATGAAGTTGGCTGTGCAGGCTTTGCATCTGTGGCTCAAGAAGAAACAGAAATTTTACTGCTGACATGGAT
 GCAGAAAGAACGCGATAAAATCATTATTACCTAACTATCTTGTGGAGTTAAAAACAGAATGCACATTGCA
 AGGAAACGAATTGTGGGAGGAAAGCGAGCACAAGTGGGAGACCTCCCATGGCAGGTGGCAATTAAGGATGCC
 AGTGAATCACCTGTGGGGGAATTTATATTGGTGGCTGTTGGATTCTGACTGCTGCACATTGTCTCAGAGCC
 AGTAAACTCATCGTTACCAATATGGACAACAGTAGTAGACTGGATACACCCCGACCTTAAACGTATAGTA
 15 ATTGAATACGTGGATAGAATTATTTCCATGAAAGTACAATGCAGGCACCTACCAAAATGACATCGCTTGT
 ATTGAATGAAAAAGACGCGAAACAAAAAGATTGTGAGCTGCCTCGTTCCATCCCTGCCTGTGTCCCTGG
 TCTCTTACCTATTCCAACCTAATGATACATGCATCGTTTCTGGCTGGGGACGAGAAAAAGATAACGAAAGA
 GTCTTTTCACTTTCAGTGGGGTGAAGTTAACTAATAAGCAACTGCTCTAAGTTTACGGAAATCGTTTCTAT
 GAAAAAGAAATGGAATGTGCAGGTACATATGATGGTTCCATCGATGCCTGTAAAGGGGACTCTGGAGGCCCC
 20 TTAGTCTGTATGGATGCCAACAATGTGACTTATGTCTGGGGTGTGTGAGTTGGGGGAAAACTGTGGAAAA
 CCAGAGTTCCAGGTGTTTACACCAAGTGGCCAATTATTTTACTGATTAGCTACCATGTAGGAAGGCTT
 TTTATTCTCAGTACAATGTATAA

(SEQ ID NO: 2)

In some embodiments, the nucleotide sequences of Complement Factor I used in the
 25 invention are codon-optimised. Different cells differ in their usage of particular codons. This
 codon bias corresponds to a bias in the relative abundance of particular tRNAs in the cell
 type. By altering the codons in the sequence so that they are tailored to match with the
 relative abundance of corresponding tRNAs, it is possible to increase expression. By the
 same token, it is possible to decrease expression by deliberately choosing codons for which
 30 the corresponding tRNAs are known to be rare in the particular cell type. Thus, an additional
 degree of translational control is available.

A preferred nucleotide sequence encoding Complement Factor I is the nucleotide sequence
 disclosed as SEQ ID NO: 10.

ATGAAACTGCTGCATGTCTTCCTCCTCTTCCTGTGCTTCCACCTCCGTTTCTGTAAAGTCACCTACACTAGC
 35 CAGGAGGATCTGGTGGAGAAGAAATGCCTGGCCAAGAAGTATACCCACCTGAGCTGCGACAAAGTGTCTGC
 CAGCCCTGGCAACGCTGCATTGAAGGTACTTGTGTGTGCAAGCTGCCCTACCAGTGCCCCAAGAAGGCACG
 GCCGTGTGTGCCACCAACAGGAGGAGCTTCCCCACCTACTGCCAGCAGAAGAGCCTGGAATGCCTCCACCCT
 GGCACCAAGTTTCTGAACAACGGGACCTGCACAGCCGAGGGGAAATTCAGCGTCTCCCTCAAGCACGGCAAT
 ACAGACTCCGAGGGCATTGTGGAAGTGAAGCTGGTGGACCAGGACAAGACCATGTTTCATCTGCAAAAGCAGC
 40 TGGTCCATGCGGGAGGCCAATGTCGCCTGCCTGGACCTGGGCTTCCAGCAGGGCGCTGATACACAGCGCCGC
 TTTAAACTCAGTGACCTCAGCATCAACAGCACTGAGTGTCTGCAAGTGCCTGCCGGGGCTGGAGACCAGC
 CTGGCTGAGTGCACCTTCACCAAGCGCAGGACCATGGGCTACCAGGATTTGTCAGATGTGGTCTGCTACACC
 CAGAAGGCAGACAGCCCCATGGATGACTTCTTCCAGTGTGTCAATGGCAAGTACATTTCCAGATGAAGGCT
 TGTGACGGGATCAATGATTGCGGGGATCAGAGCGATGAGCTCTGCTGCAAGGCTGCCAAGGGAAGGGCTTT
 45 CACTGTAAGTCTGGGGTGTGCATCCCTTCTCAGTATCAGTGCACGGAGAGGTGGACTGCATCACTGGGGAG
 GACGAGTGGGCTGTGCTGGCTTCGCCTCTGTGGCCAGGAGGAGACAGAGATCCTCACAGCTGACATGGAT
 GCAGAGCGGCGGCATCAAGAGTCTGCTCCCAAAGCTTCTCTGCGGCGTTAAGAATCGCATGCACATCCGG
 AGGAAGCGGATCGTTGGAGGCAAACGGGCTCAGCTGGGGGACTTGGCTGGCAGGTGGCCATCAAGATGCC
 50 TCCGGAATCACCTGTGGTGGCATCTACATCGGCGGCTGCTGGATCCTGACCGCGGCCACTGCTTCGGGCC
 AGCAAGACTACCGCTACCAGATCTGGACCACCGTGGTGGATTGGATTACCCCGACCTGAAGAGGATTGTC

ATTGAGTATGTCGACCGCATCATCTTCCATGAAAACTACAATGCCGGGACGTATCAGAACGACATCGCCCTC
 ATCGAGATGAAGAAGGATGGGAACAAGAAGGACTGTGAGCTGCCTCGCTCCATCCCCGCCTGTGTACCATGG
 TCTCCGTACCTGTTCCAGCCAAATGACACATGCATCGTGAGCGGCTGGGGCCGCGAGAAAGACAACGAGAGG
 GTCTTCTCCCTGCAGTGGGGTGAAGTCAAGCTGATCAGCAACTGCTCCAAGTTCTACGGCAACCGCTTCTAT
 5 GAGAAGGAGATGGAGTGCGCCGGCACCTATGACGGCAGCATTGACGCGTGCAAGGGAGACAGTGGGGGCCCC
 CTGGTCTGCATGGACGCCAACAAATGTGACCTACGTGTGGGGAGTTGTGTCTGGGGCGAGAACTGTGGCAAG
 CCTGAGTTCCCGGGCGTGTACACAAAGGTGGCAAATATTTTGAAGTGGATCTCCTATCACGTTGGCAGGCCC
 TTCATTTACAGTACAACGTATAA

(SEQ ID NO: 10)

- 10 A further example codon-optimised nucleotide sequence encoding Complement Factor I is
 SEQ ID NO: 8.

ATGAAGCTGCTGCATGTCTTTCTGCTGTTTCTGTGCTTCCATCTGCGGTTCTGTAAAGTGACCTATACTAGC
 CAGGAGGATCTGGTGGAGAAGAAGTGTCTGGCCAAGAAGTACACACACCTGAGCTGCGACAAGGTGTTCTGT
 CAGCCTTGGCAGCGGTGCATCGAGGGCACCTGCGTGTGCAAGCTGCCTTACCAGTGCCCAAAGAAGCGCACC
 15 GCCGTGTGCGCCACAAATCGGAGATCTTTTCCAACATATTGCCAGCAGAAGAGCCTGGAGTGTCTGCACCCC
 GGCACCAAGTTCTTGAACAATGGCACCTGCACAGCCGAGGGCAAGTTTTCTGTGAGCCTGAAGCACGGCAAC
 ACAGATAGCGAGGGCATCGTGGAGGTGAAGCTGGTGGACCAGGATAAGACCATGTTTATCTGTAAGAGCTCC
 TGGTCCATGAGGGAGGCAACCGTGGCATGCCTGGATCTGGGATTCCAGCAGGGAGCAGACACACAGAGGCGC
 20 TTTAAGCTGTCCGACCTGTCTATCAATAGCACCGAGTGCCTGCACGTGCACGTAGGGGCTGGAGACATCC
 CTGGCAGAGTGCACCTTCACAAAGCGGAGAACCATGGGCTACCAGGACTTTGCCGACGTGGTGTCTATACC
 CAGAAGGCCGATAGCCCATGGACGATTTCTTTAGTGCCTGAACGGCAAGTATATCTCCAGATGAAGGCC
 TGCGACGGCATCAATGACTGTGGCGATCAGTCTGACGAGCTGTGCTGTAAGGCCTGTGAGGGCAAGGGCTTC
 CACTGCAAGAGCGGCGTGTGCATCCCTTCCCAGTACCAGTGCAACGGCGAGGTGGATTGTATCACAGGAGAG
 25 CACGAAGTGGGATGCCGAGGATTTGCATCTGTGGCACAGGAGGAGACAGAGATCCTGACAGCCGACATGGAT
 GCCGAGAGGGCGCCGGATCAAGTCTCTGCTGCCTAAGCTGAGCTGTGGCGTGAAGAATCGGATGCACATCAGA
 AGGAAGCGCATCGTGGGAGGCAAGAGGGCACAGCTGGGCGATCTGCCATGGCAGGTGGCCATCAAGGACGCC
 TCTGGCATCACCTGCGCGGCATCTACATCGGAGGATGTTGGATCCTGACCGCAGCACACTGCCTGAGAGCA
 AGCAAGACACACAGGTATCAGATCTGGACCACAGTGGTGGATTGGATCCACCCAGACCTGAAGAGAATCGTG
 30 ATCGAGTACGTGGATAGGATCATCTTTCACGAGAACTACAATGCCGGCACATATCAGAACGACATCGCCCTG
 ATCGAGATGAAGAAGGATGGCAATAAGAAGGACTGTGAGCTGCCCAGATCCATCCCTGCATGCGTGCCATGG
 AGCCCCATCTGTTCCAGCCCAACGATACCTGCATCGTGTCCGGATGGGGAAGGGAGAAGGACAATGAGCGG
 GTGTTTTCTCTGCAGTGGGGCGAGGTGAAGCTGATCTCCAACGTTTCTAAGTTCTACGGCAATAGGTTTTAT
 GAGAAGGAGATGGAGTGCGCCGGCACCTACGATGGCAGCATCGACGCTGTAAGGGCGATTCCCGAGGACCA
 35 CTGGTGTGCATGGACGCAACAATGTGACATACGTGTGGGGAGTGGTGTCTGGGGAGAGAACTGCGGCAAG
 CCAGAGTTCCCGGCGTATATACCAAGGTGGCAATTATTTTGATTGGATTTCCTACCACGTGCGCAGGCCCC
 TTTATTTCCAGTATAATGTCTAA

(SEQ ID NO: 8)

- In some embodiments, the nucleotide sequence encoding Complement Factor I has at least
 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%
 40 or 99% identity to SEQ ID NO: 10, 8 or 2, preferably SEQ ID NO: 10. Preferably, the protein
 encoded by the nucleotide sequence substantially retains a functional activity of the protein
 represented by SEQ ID NO: 1 or 9.

In other embodiments, the nucleotide sequence encoding Complement Factor I is SEQ ID
 NO: 10, 8 or 2, preferably SEQ ID NO: 10.

- 45 In other embodiments, the nucleotide sequence encoding Complement Factor I has at least
 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to

positions 55 to 1752 of SEQ ID NO: 10, 8 or 2, preferably SEQ ID NO: 10. Preferably, the protein encoded by the nucleotide sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 1 or 9.

5 In other embodiments, the nucleotide sequence encoding Complement Factor I is positions 55 to 1752 of SEQ ID NO: 10, 8 or 2, preferably SEQ ID NO: 10.

In other embodiments, the nucleotide sequence encoding Complement Factor I encodes an amino acid sequence that has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 1 or 9. Preferably, wherein the amino acid sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 1 or 9.

10 In other embodiments, the nucleotide sequence encoding Complement Factor I encodes the amino acid sequence SEQ ID NO: 1 or 9.

In other embodiment, the nucleotide sequence encoding Complement Factor I encodes an amino acid sequence that has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to positions 19 to 583 of SEQ ID NO: 1 or 9. Preferably, wherein the amino acid
15 sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 1 or 9.

In other embodiments, the nucleotide sequence encoding Complement Factor I encodes the amino acid sequence of positions 19 to 583 of SEQ ID NO: 1 or 9.

An advantage of the invention is that Complement Factor I is particularly difficult to prepare
20 in the form of a purified protein. Accordingly, the inventors have devised a way of modulating the complement system, for example to enable treatments of age-related macular degeneration (AMD), by administering Complement Factor I in the form of an AAV vector comprising a Complement Factor I-encoding nucleotide sequence. The AAV vector may be administered to a site of interest, for example the eye, to enable in situ translation of the
25 Complement Factor I polypeptide.

COMPLEMENT FACTOR I (CFI) COFACTOR

The term "Complement Factor I (CFI) cofactor", as used herein, may refer to a protein that is capable of acting as a cofactor for the CFI-mediated cleavage of C3b.

In some embodiments, the Complement Factor I (CFI) cofactor is selected from the group
30 consisting of Complement Factor H Like Protein 1 (FHL1); Complement Factor H (CFH);

Complement Receptor 1 (CR1) or a fragment thereof; and Membrane Cofactor Protein (MCP) or a fragment thereof.

- In some embodiments, the Complement Factor I (CFI) cofactor is Complement Factor H (CFH). In some embodiments, the Complement Factor I (CFI) cofactor is Complement Receptor 1 (CR1) or a fragment thereof. In some embodiments, the Complement Factor I (CFI) cofactor is Membrane Cofactor Protein (MCP) or a fragment thereof.

In preferred embodiments, the Complement Factor I (CFI) cofactor is Complement Factor H Like Protein 1 (FHL1).

COMPLEMENT FACTOR H (CFH)

- Complement Factor H (Factor H, CFH) is a complement control protein.

Complement Factor H is a large (155 kDa), soluble glycoprotein that is present in human plasma at a typical concentration of 200–300 µg/mL (Hakobyan et al. (2008) 49(5): 1983-90). The principal function of Complement Factor H is to regulate the alternative pathway of the complement system.

- Complement Factor H provides cofactor activity for the Complement Factor I-mediated cleavage of C3b. Complement Factor H also increases the rate of dissociation of the C3bBb complex (C3 convertase) and the (C3b)NBb complex (C5 convertase) and thereby reduces the activity of the alternative complement pathway.

- Complement Factor H is made up of 20 complement control protein (CCP) modules (also referred to as Short Consensus Repeats or sushi domains) connected to one another by short linkers (of between three and eight amino acid residues) and arranged in an extended head to tail fashion. Each of the CCP modules consists of around 60 amino acids with four cysteine residues disulfide bonded in a 1-3 2-4 arrangement, and a hydrophobic core built around an almost invariant tryptophan residue. The CCP modules are numbered from 1-20 (from the N-terminus of the protein). CCPs 1-4 and CCPs 19-20 engage with C3b while CCPs 6-8 and CCPs 19-20 bind to GAGs and sialic acid (Schmidt et al. (2008) Journal of Immunology 181: 2610–2619).

- It has been shown that gene therapy using Complement Factor H can ameliorate induced AMD-like pathology in mice (Cashman et al. (2015) J. Gene Med. 17: 229-243). Mice were co-injected subretinally with: (i) an adenoviral vector expressing complement component C3, which had previously been shown to recapitulate many pathological features of human AMD; and (ii) an adenoviral vector expressing Complement Factor H. Relative to control animals

receiving GFP instead of Complement Factor H, the Complement Factor H-transduced mice showed 91% reduction in endothelial cell proliferation and 69% attenuation of RPE atrophy. Electoretinography showed improved retinal function in mice receiving Complement Factor H, and immunocytochemistry of rhodopsin and RPE65 was consistent with the rescue of photoreceptors and RPE in such animals.

- In some embodiments, a Complement Factor H polypeptide or a fragment or derivative thereof is capable of acting as a cofactor for the Complement Factor I-mediated cleavage of C3b. In some embodiments, a Complement Factor H polypeptide or a fragment or derivative thereof is capable of increasing the rate of dissociation of C3 convertase and C5 convertase.
- 10 In preferred embodiments, a Complement Factor H polypeptide or a fragment or derivative thereof is capable of acting as a cofactor for the Complement Factor I-mediated cleavage of C3b and increasing the rate of dissociation of C3 convertase and C5 convertase.

In some embodiments, the Complement Factor H is human Complement Factor H.

- An example human Complement Factor H protein is the human Complement Factor H protein having the UniProtKB accession number P08603. This exemplified sequence is 1231 amino acids in length (disclosed as SEQ ID NO: 3) of which amino acids 1 to 18 form a signal sequence.

- In some embodiments, the amino acid sequence of Complement Factor H is SEQ ID NO: 3. In other embodiments, the amino acid sequence of Complement Factor H is positions 19 to 1231 of SEQ ID NO: 3.

25 MRLAKIICLMLWAICVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPGYRSLGNVIMVCRKGEWV
ALNPLRKCQKRPCGHPGDTPTFGTFTLTGGNVFEYGVKAVYTCNEGYQLLGEINYRECDTDCGTNDIPICEVV
KCLPVTAPENGKIVSSAMEPDREYHFGQAVRFVNCNSGYKIEGDEEMHCSDDGFWSEKPKCIVEISCKSPDVI
NGSPISQKIIYKENERFYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSPLRIKHRTGD
EITYQCRNGFYPATRGNTAKCTSTGWIPAPRCTLKPCDYPDIKHGGLYHENMRRPYFPVAVGKYYSYYCDEH
FETPSGSYWDHIHCTQDGWSPAVPCLRKCYFPYLENGYNQNYGRKFVQGSIDVACHPGYALPKAQTTVTCM
ENGWSPTPRCIRVKTCSKSSIDIENGFISESQYTYALKEKAKYQCKLGYVTADGETSGSITCGKDGWSAQPT
CIKSCDIPVFMNARTKNDFTWFKLNDTLDYECHDGYESNTGSTTGSIVCGYNGWSDLPICYERECELPKIDV
HLVPDRKKDQYKVGVLKFSCKPGFTIVGPNVQCYHFGLSPLPICKEQVQSCGPPPELLNGNVKEKTKEE
30 YGHSEVVEYYCNPRFLMKGPNKIQCVDEWTTLPVCIVEESTCGDIPELEHGWAQLSSPPYYYGDSVEFNCS
ESFTMIGHRSITCIHGVTQLPQCAIDKLKKCKSSNLIILEEHLKNKKEFDHNSNIRYRCRGKEGWIHTVC
INGRWDPEVNCMAQIQLCPPPPQIPNSHNMTTLNRYDGEKVSVLCQENYLIQEGEEITCKDGRWQSIPLC
VEKIPCSQPPQIEHGTINSSRSSQESYAHGTLKLSYTCGGFRISEENETTCYMGKWSSPPQCEGLPCKSPPE
ISHGVVAHMSDSYQYGEEVYKCFEGFGIDGPAIAKCLGEKWSHPPSCIKTDCLSLPSFENAIPMGEKKDVY
35 KAGEQVITYTCATYYKMDGASNVTICNSRWTGRPTCRDTSCVNPPTVQNAVIVSRQMSKYPSEGERVRYQCRSP
YEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPIDNGDITSFPLSVYAPASSVEYQCQNLQLEGNKRITC
RNGQWSEPPKCLHPCVISREIMENYNIALRWTAQKLYSRTGESVEFVCKRGYRLSSRSHTLRTTCWDGKLE
YPTCAKR

(SEQ ID NO: 3)

An example nucleotide sequence encoding Complement Factor H is the nucleotide sequence having the NCBI Accession No. NM_000186.

In some embodiments, the nucleotide sequence encoding Complement Factor H is SEQ ID NO: 4.

```
5  ATGAGACTTCTAGCAAAGATTATTTGCCCTTATGTTATGGGCTATTTGTGTAGCAGAAGATTGCAATGAACCTT
   CCTCCAAGAAGAAATACAGAAATTTCTGACAGGTTCTGGTCTGACCAAACATATCCAGAAGGCACCCAGGCT
   ATCTATAAATGCCGCCCTGGATATAGATCTCTTGGAAATGTAATAATGGTATGCAGGAAGGGAGAATGGGTT
   GCTCTTAATCCATTAAAGAAATGTCAGAAAAGGCCTGTGGACATCCTGGAGATACTCCTTTTGGTACTTTT
10  ACCCTTACAGGAGGAAATGTGTTTGAATATGGTGTAAAAGCTGTGTATACATGTAATGAGGGGTATCAATTG
   CTAGGTGAGATTAATTACCGTGAATGTGACACAGATGGATGGACCAATGATATTCCTATATGTGAAGTTGTG
   AAGTGTTTACCAGTGACAGCACCAGAGAATGGAAAAATTGTGCTAGTGCATGGAACCAGATCGGGAATAC
   CATTTTGGACAAGCAGTACGGTTTGTATGTAACCTCAGGCTACAAGATTGAAGGAGATGAAGAAATGCATTGT
   TCAGACGATGGTTTTTGGAGTAAAGAGAAACCAAGTGTGTGGAATTTTCATGCAATCCCAGATGTTATA
15  AATGGATCTCCTATATCTCAGAAGATTATTTATAAGGAGAATGAACGATTTCAATATAAATGTAACATGGGT
   TATGAATACAGTGAAAGAGGAGATGCTGTATGCACTGAATCTGGATGGCGTCCGTTGCCCTTCATGTGAAGAA
   AAATCATGTGATAATCCTTATATTTCCAAATGGTGACTACTCACCTTTAAGGATTAAACACAGAACTGGAGAT
   GAAATCAGGTACCGTGTAGAAATGGTTTTTATCCTGCAACCCGGGGAAATACAGCAAAATGCACAAGTACT
   GGCTGGATACCTGCTCCGAGATGTACCTTGAACCTTGTGATTATCCAGACATTAAACATGGAGGTCTATAT
20  CATGAGAATATGCGTAGACCATACTTTCCAGTAGCTGTAGGAAAAATATTACTCCTATTACTGTGATGAACAT
   TTTGAGACTCCGTCAGGAAGTTACTGGGATCACATTCATTGCACACAAGATGGATGGTCGCCAGCAGTACCA
   TGCCTCAGAAAATGTTATTTTCCCTTATTTGGAAAAATGGATATAATCAAAATCATGGAAGAAAGTTTGTACAG
   GGTAATCTATAGACGTTGCCCTGCCATCCTGGCTACGCTCTTCCAAAAGCGCAGACCACAGTTACATGTATG
   GAAATGGCTGGTCTCCTACTCCCAGATGCATCCGTGTCAAAACATGTTCCAAATCAAGTATAGATATTGAG
25  AATGGGTTTATTTCTGAATCTCAGTATACATATGCCTTAAAAGAAAAAGCGAAATATCAATGCAAACTAGGA
   TATGTAACAGCAGATGGTGAAACATCAGGATCAATTACATGTGGGAAAGATGGATGGTCAGCTCAACCCACG
   TGCATTAATCTTGTGATATCCCAGTATTTATGAATGCCAGAACTAAAAATGACTTCACATGGTTTTAAGCTG
   AATGACACATTGGACTATGAATGCCATGATGGTTATGAAAGCAATACTGGAAGCACCCTGGTTCATAGTG
   TGTGGTTACAATGGTTGGTCTGATTTACCCATATGTTATGAAAGAGAATGCGAACTTCTTAAATAGATGTA
30  CACTTAGTTCTGATCGCAAGAAAGACCAGTATAAAGTTGGAGAGGTGTTGAAATTTCTCTGCAAAACCAGGA
   TTTACAATAGTTGGACCTAATTCCGTTCCAGTGTACCACTTTGGATTGTCTCCTGACCTCCCAATATGTAAA
   GAGCAAGTACAATCATGTGGTCCACCTCCTGAACTCCTCAATGGGAATGTTAAGGAAAAAACGAAAGAAGAA
   TATGGACACAGTGAAGTGGTGGAATATTATTGCAATCCTAGATTTCTAATGAAGGGACCTAATAAAATTCAA
   TGTGTTGATGGAGAGTGGACAACCTTTACCAGTGTGATTGTGGAGGAGAGTACCTGTGGAGATATACCTGAA
35  CTTGAACATGGCTGGGCCAGCTTTCTTCCCCTCCTTATTACTATGGAGATTCAGTGGAAATCAATTGCTCA
   GAATCATTTTACAATGATTGGACACAGATCAATTACGTGTATTTCATGGAGTATGGACCAACTTCCCAGTGT
   GTGGCAATAGATAAACTTAAGAAGTGCAATCATCAAATTTAATTATACTTGAGGAACATTTAAAAAACAAAG
   AAGGAATTCGATCATAATTCTAACATAAGGTACAGATGTAGAGGAAAAGAAGATGGATACACACAGTCTGC
   ATAAATGGAAGATGGGATCCAGAAGTGAAGTGCCTCAATGGCACAATAACAATTATGCCACCTCCACCTCAG
40  ATTCCCAATTCTCACAATATGACAACCACACTGAATTATCGGGATGGAGAAAAAGTATCTGTTCTTTGCCAA
   GAAAATTATCTAATTCAGGAAGGAGAAGAAATTACATGCAAAGATGGAAGATGGCAGTCAATACCCTCTGT
   GTTGAAAAAATTCATGTTTACAACCACCTCAGATAGAACACGGAACCATTAATTCATCCAGGTCTTCACAA
   GAAAGTTATGCACATGGGACTAAATTGAGTTATACTTGTGAGGGTGGTTTTCAGGATATCTGAAGAAAATGAA
   ACAACATGCTACATGGGAAAATGGAGTTCTCCACCTCAGTGTGAAGGCCTTCCCTGTAAATCTCCACCTGAG
45  ATTTCTCATGGTGTGTAGCTCACATGTCAGACAGTTATCAGTATGGAGAAGAAGTTACGTACAAATGTTTT
   GAAGGTTTTGGAATTTGATGGGCCCTGCAATTGCAAAATGCTTAGGAGAAAAATGGTCTCACCCTGCATGTG
   ATAAAAACAGATTTGCTCAGTTTACCTAGTTTGAAGATGCCATACCCATGGGAGAGAGAGGATAGGATGAT
   AAGGCGGGTGAGCAAGTGACTTACACTTGTGCAACATATTACAAAATGGATGGAGCCAGTAATGTAACATGC
   ATTAATAGCAGATGGACAGGAAGGCCAACATGCAGAGACACCTCCTGTGTGAATCCGCCCACAGTACAAAAAT
50  GCTTATATAGTGTGAGACAGATGAGTAAATATCCATCTGGTGAGAGAGTACGTTATCAATGTAGGAGCCCT
   TATGAAATGTTTTGGGGATGAAGAAGTGATGTGTTAAATGGAACTGGACGGAACACCTCAATGCAAGAT
   TCTACAGGAAAATGTGGGCCCCCTCCACCTATTGACAAATGGGGACATTACTTCATTTCCGTTGTGCTAGTATAT
   GCTCCAGTTTACAGTTGAGTACCAATGCCAGAACTTGATCAACTGAGGGTAAACAAGCGAATAACATGAT
   AGAAATGGACAATGGTCAGAACCAACCAAAATGCTTACATCCGTGTGTAATATCCCGAGAAATATGGAAAAAT
55  TATAACATAGCATTAAAGGTGGACAGCCAAACAGAAGCTTTATTCGAGAACAGGTGAATCAGTTGAATTTGTG
   TGTAACGGGGATATCGTCTTTCATCACGTTCTCACACATTGCGAACAACATGTTGGGATGGGAACTGGAG
   TATCCAACCTGTGCAAAAAGATAG
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(SEQ ID NO: 4)

In some embodiments, the nucleotide sequence encoding Complement Factor H has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 4. Preferably, wherein the protein encoded by the nucleotide sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 3.

In other embodiments, the nucleotide sequence encoding Complement Factor H is SEQ ID NO: 4.

In other embodiments, the nucleotide sequence encoding Complement Factor H has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to positions 55 to 3696 of SEQ ID NO: 4. Preferably, wherein the protein encoded by the nucleotide sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 3.

In other embodiments, the nucleotide sequence encoding Complement Factor H is positions 55 to 3696 of SEQ ID NO: 4.

In other embodiments, the nucleotide sequence encoding Complement Factor H encodes an amino acid sequence that has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 3. Preferably, wherein the amino acid sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 3.

In other embodiments, the nucleotide sequence encoding Complement Factor H encodes the amino acid sequence SEQ ID NO: 3.

In other embodiment, the nucleotide sequence encoding Complement Factor H encodes an amino acid sequence that has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to positions 19 to 1231 of SEQ ID NO: 3. Preferably, wherein the amino acid sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 3.

In other embodiments, the nucleotide sequence encoding Complement Factor H encodes the amino acid sequence of positions 19 to 1231 of SEQ ID NO: 3.

COMPLEMENT FACTOR H-LIKE PROTEIN 1 (FHL1)

Complement Factor H-like Protein 1 (FHL1) is a splice variant of Complement Factor H that contains the first 7 CCPs of Complement Factor H followed by a four amino acid carboxy-terminal tail (Clark, S.J. et al. (2015) J Clin Med 4: 18-31).

In some embodiments, the FHL1 is human FHL1.

In some embodiments, the amino acid sequence of FHL1 is SEQ ID NO: 11.

5 MRLAKIICMLWAI CVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPGYRSLGNIIMVCRKGEWV
ALNPLRKQCQRPCGHPGDTFPGTFTLTGGNVFEYGVKAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVV
KCLPVTAPENGKIVSSAMEPDREYHFGQAVRFVCSNGYKIEGDEEMHCSDDGFWSKEPKCWEISCKSPDVI
NGSPISQKIIYKENERFQYKCNMGYEYSERGDVCTESGWRPLPSCEEKSCDNPIYPNGDYSPRLIKHRTGD
EITYQCRNGFYPATRGNTAKCTSTGWIPAPRCTLKPCDYPDIKHGGLYHENMRRPYFPVAVGKYYSYCDHEH
FETPSGSYWDHIHCTQDGWSPAVPLRKCYFPYLENGYNQNYGRKFVQGKSIDVACHPGYALPKAQTTVTCM
ENGWSPTPRCIRVSFTL

10 (SEQ ID NO: 11)

An example nucleotide sequence encoding FHL1 is:

15 ATGAGACTTCTAGCAAAGATTATTTGCCTTATGTTATGGGCTATTTGTGTAGCAGAAGATTGCAATGAACTT
CCTCCAAGAAGAAATACAGAAATTCTGACAGGTTCTGGTCTGACCAAACATATCCAGAAGGCACCCAGGCT
ATCTATAAATGCCGCCCTGGATATAGATCTCTTGGAAATATAATAATGGTATGCAGGAAGGGAGAATGGGTT
GCTCTTAATCCATTAAGGAAATGTCAGAAAAGGCCCTGTGGACATCCTGGAGATACTCCTTTTGGTACTTTT
ACCCTTACAGGAGGAAATGTGTTTGAATATGGTGTAAAAGCTGTGTATACATGTAATGAGGGGTATCAATTG
CTAGGTGAGATTAATTACCGTGAATGTGACACAGATGGATGGACCAATGATATTCCTATATGTGAAGTTGTG
AAGTGTTTACCAAGTGACAGCACCAGAGAATGGAAAAATTGTCTAGTAGTCAATGGAACCAGATCGGGAATAC
CATTTTGGACAAGCAGTACGGTTTGTATGTAACCTCAGGCTACAAGATTGAAGGAGATGAAGAAATGCATTGT
20 TCAGACGATGGTTTTTGGAGTAAAGAGAAACCAAAGTGTGTGGAAATTTTCATGCAAATCCCCAGATGTTATA
AATGGATCTCCTATATCTCAGAAGATTATTTATAAGGAGAATGAACGATTTCAATATAAATGTAACATGGGT
TATGAATACAGTGAAAGAGGAGATGCTGTATGCACTGAATCTGGATGGCGTCCGTTGCCTTCATGTGAAGAA
AAATCATGTGATAATCCTTATATTTCAAATGGTGACTACTCACCTTTAAGGATTAAACACAGAAGTGGAGAT
25 GAAATCACGTACCAGTGTAGAAATGGTTTTTATCCTGCAACCCGGGGAAATACAGCaAAATGCACAAGTACT
GGCTGGATACCTGCTCCGAGATGTACCTTGAACCTTGTGATTATCCAGACATTAACATGGAGGCTATAT
CATGAGAATATGCGTAGACCATACTTTCCAGTAGCTGTAGGAAAATATTACTCCTATTACTGTGATGAACAT
TTTGAGACTCCGTCAGGAAGTTACTGGGATCACATTCAATGCACACAAGATGGATGGTGCACGAGCAGTACCA
TGCCTCAGAAAATGTTATTTTCCCTTATTTGGAAAATGGATATAATCAAAATATGGAAGAAAGTTTGTACAG
30 GGTAATCTATAGACGTTGCCTGCCATCCTGGCTACGCTCTTCCAAAAGCGCAGACCACAGTTACATGTATG
GAGAATGGCTGGTCTCCTACTCCAGATGCATCCGTGTGAGCTTTACCCCTCTGA

(SEQ ID NO: 16)

The nucleotide sequences of FHL1 used in the invention are preferably codon optimised.

A preferred nucleotide sequence encoding FHL1 is SEQ ID NO: 12.

35 ATGCGCCTCCTGGCCAAGATCATCTGCCTCATGCTGTGGGCCATCTGCGTGGCTGAGGACTGCAATGAGCTG
CCGCCCAGGAGGAACACAGAGATCCTGACAGGGAGCTGGTCTGACCAGACCTACCCTGAGGGCACCCAGGCG
ATCTACAAGTGCCGGCCGGGTACAGGAGCCTGGGGAACATCATCATGGTGTGTAGAAAGGGCGAATGGGTG
GCCCTCAACCCCTGAGGAAGTCCAGAAGCGGCCCTGTGGCCACCCGGGGACACACCTTCGGGACCTTC
ACCCTGACCGGCGGCAATGTGTTTGAATACGGCGTGAAGGCTGTCTACACATGCAACGAGGGGTACCAGCTG
40 CTGGGCGAGATTAATACCGGGAGTGTGACACCGATGGGTGGACCAACGACATTCCCATCTGTGAGGTGGTC
AAGTGTCTCCCGTGACAGCCCCAGAAAATGGCAAAATCGTGAGCAGCGCCATGGAGCCTGACCGCGAATAT
CACTTTGGGCAGGCCGTGAGGTTTGTGTGCAACTCGGGCTACAAAATTGAAGGTGATGAGGAGATGCACTGC
AGCGATGATGGCTTCTGGTCCAAGGAGAAGCCCAAATGTGTGGAGATCTCCTGCAAGTCTCCCGACGTGATC
AACGGCAGCCCAATCAGCCAGAAGATTATTTACAAAGAGAACGAGCGCTTCCAGTACAAGTGAACATGGGC
TATGAGTATTAGAGAGGGGAGATGCCGTCTGCACTGAGAGCGGCTGGAGACCACTGCCTAGCTGCGAGGAA
45 AAGAGTTGTGACAACCTTACATCCCAAATGGCGACTACTCCCTCTGCGGATCAAACACCGGACCGGGGAT
GAAATCACTTACAGTGCAGCAATGGATTCTACCCGGCCACCCGGCAACACCGCAAAATGCACACGACACA
GGCTGGATCCCCCGCCCCCGCTGTACGCTGAAGCCTTGCAGCTATCCAGACATCAAGCAGGAGGCTGTAC
CACGAAAACATGCGGCGGCCTTATTTCCCTGTGGCAGTGGGGAAGTACTACAGCTACTACTGCGACGAGCAC

TTCGAGACCCCTCTGGCTCCTACTGGGACCACATCCACTGCACACAGGACGGCTGGTCTCCAGCTGTGCCC
 TGCCTGAGGAAATGCTACTTCCCCCTACCTGGAGAACGGATACAACCAGAACTATGGCCGCAAGTTCGTGCAG
 GGCAAGAGCATCGATGTGGCCTGCCACCCTGGCTACGCCCTGCCCAAGGCCAGACAACCTGTGACCTGCATG
 GAGAATGGTTGGAGCCCCACCCGCGCTGCATCCGGGTGTCCTTCACGCTCTGA

5 (SEQ ID NO: 12)

In some embodiments, the nucleotide sequence encoding FHL1 has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 12 or 16, preferably SEQ ID NO: 12. Preferably, the protein encoded by the nucleotide sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 10 11.

In other embodiments, the nucleotide sequence encoding FHL1 is SEQ ID NO: 12 or 16, preferably SEQ ID NO: 12.

COMPLEMENT RECEPTOR 1 (CR1)

Complement Receptor 1 (CR1), also known as CD35, is a type I membrane-bound 15 glycoprotein belonging to the regulators of complement activity (RCA) family. CR1 may be found on the plasma membrane of erythrocytes, eosinophils, monocytes, macrophages, B-lymphocytes, a subpopulation of CD4+ T cells, dendritic cells, Langerhan cells in the skin and glomerular podocytes.

CR1 is an ~200 kDa, single-chain glycoprotein, the extracellular portion of which comprises 20 30 complement-control-protein repeats (CCPs) or short consensus repeats. A non-membrane bound soluble form of CR1 (sCR1) is found in plasma. It may be generated by release from leukocytes by cleavage of the surface form of CR1. The structure of CR1 and sCR1 is described, for example, in Liu, D. et al. (2009) Immunopharmacology and Immunotoxicology 31: 524–535.

25 In some embodiments, the CR1 or fragment thereof is human CR1 or a fragment thereof.

An example CR1 sequence is:

MGASSPRSPPEVGPAPGLPFCCGGSLLAVVLLALPVAWGQCNAPEWLPFARPTNLTDEFEFFIGTYLNYE
 CRPGYSGRPFSSIICLNKNSVWTGAKDRCKRKRSCRNPPDPVNGMVHVIKGIQFGSQIKYSCTKGYRLIGSSSAT
 30 CIISGDTVIWDNETPICDRIPCGLPPTITNGDFISTNRENFHYGSVVTYRCNPGSGGRKVFELVGEPSIYCT
 SNDDQVGIIWGPAPQCIIPNKCTPPNVENGILVSDNRSLSLNEVVEFRCPGFVMKGPRRVKQCQALNKWEP
 ELPSCSRVCQPPPDVLHAERTQRDKDNFSPGQEVFYSCPEGYDLRGAASMRCTPQGDWSPAAPTCEVKSDD
 FMGQLLNGRVLFVNLQLGAKVDFVCDGFLKGSSASYCVLAGMESLWNSSVPVCEQIFCPSPPIPNGRH
 TGKPLEVFPFGKTVNYTCDPHPDGRGTSFDLIGESTIRCTSDPQNGVWSSPAPRCGILGHCQAPDHFLFAKL
 35 KTQTNASDFPIGTSKYECPREYYGRPFSITCLDNLVWSSPKDVCKRKSKCTPPDPVNGMVHVIDIQVGSR
 INYSCTTGHRLLIGHSSAECILSGNAAHWSTKPPICQRIPCGLPPTIANGDFISTNRENFHYGSVVTYRCNPG
 SSGRKVFELVGEPSIYCTSNDDQVGIIWGPAPQCIIPNKCTPPNVENGILVSDNRSLSLNEVVEFRCPGF
 VMKGPRRVKQCQALNKWEPFLPSCSRVCQPPPDVLHAERTQRDKDNFSPGQEVFYSCPEGYDLRGAASMRCTP
 QGDWSPAAPTCEVKSDDFMGQLLNGRVLFVNLQLGAKVDFVCDGFLKGSSASYCVLAGMESLWNSSVP

VCEQIFCPSPVPVINGRHTGKPLEVFPFGKAVNYTCDPHDPDRGTSFDLIGESTIRCTSDPQNGVWSSPAPR
 CGILGHCQAPDHFLFAKLKTQTNASDFPIGTSKLYECRPEYYGRPFSITCLDNLVWSSPKDVCKRKSKCTPP
 DPVNGMVHVITDIQVGSRIYNSCTTGHRLLIGHSSAECILSGNTAHWSTKPPICQRI PCGLPPTIANGDFIST
 NRENFHYGVSVVTYRCNLGSRGRKFVFLVGEPSIYCTSNDDQVGIWSGPAPQCIIPNKCTPPNVENGILVSDN
 5 RSLFSLNEVVEFRCPQGFVMKGP RRVKCQALNKWEPPELPSCSRVCQPPPEILHGEHTPSHQDNFSPGQEVFY
 SCEPGYDLRGAASLHCTPQGDWSPEAPRCVAVKSCDDFLGQLPHGRVLFPLNLQLGAKVSFVCDGFRKLGSS
 VSHCVLVGMRSLWNNVVPVCEHIFCPNPPAILNGRHTGTPSGDIPYKEISYTCDPHPDRGMTFNLIGESTI
 RCTSDPHGNGVWSSPAPRCELSVRAGHCKTPEQFPFASPTIPINDFEFPVGTSLNYECRPGYFGKMFISISCL
 10 ENLVWSSVEDNCRKSKCGPPPEPFNGMVHINTDTQFGSTVNYSCNEGFRLLIGSPSTTCLVSGNNVTWDKKAP
 ICEIISCEPPPTISNGDFYSNNRTSFHNGTVVTYQCHTGP DGEQLFELVGERSIYCTSKDDQVGVWSSPPPR
 CISTNKCTAPEVENAIRVPGNRSSFTLTETIRFRCQPGFVMVGSHTVQCQTNGRWGPKLPHCSRVCQPPPEI
 LHGEHTLSHQDNFSPGQEVFYSCPSYDLRGAASLHCTPQGDWSPEAPRCTVKSCDDFLGQLPHGRVLLPLN
 LQLGAKVSFVCDGFRKLGSRASHCVLAMKALWNNVVPVCEQIFCPNPPAILNGRHTGTPFGDIPYKEIS
 15 YACDTHPDRGMTFNLIGESSIRCTSDPQNGVWSSPAPRCELSVPAACPHPPKIQNGHYIGGHVSLYLPGMT
 ISYICDPGYLLVGKGFIFCTDQGIWSQLDHYCKEVNCSFPLFMNGISKELEMKKVYHYGDYVTLKCEDGYTL
 EGSPWSQCQADDRWDPLAKCTSRTHDALIVGTLSGTIFFILLIIFLSWIIILKHRKGNNAHENPKEVAIHLH
 SQGGSSVHPRTLQTNENSRVLP

 (SEQ ID NO: 24)

An example nucleotide sequence encoding CR1 is:

20 ATGGGGGCTCTTCTCCAAGAAGCCCGGAGCCTGTGCGGGCCGCGGCGCCCGGTCTCCCCTTCTGCTGCGGA
 GGATCCCTGCTGGCGGTGTGGTGTCTGCTTGCCTGCGGTGGCCTGGGGTCAATGCAATGCCCCAGAAATGG
 CTTCCATTGCGAGGCTACCAACCTAACTGATGAATTTGAGTTTCCATTGGGACATATCTGAAGTATGAA
 TGCCGCGCTGTTTATTCGGAAGACCGTTTTCTATCATCTGCCTAAAAAATCAGTCTGGACTGGTGTAAAG
 GACAGGTGCAGACGTAAATCATGTCTGTAATCCTCCAGATCCTGTGAATGGCATGGTGCATGTGATCAAAGGC
 25 ATCCAGTTCCGATCCCAAATTAATATTCTTGTACTAAAGGATACCGACTCATTGGTTCCTCGTCTGCCACA
 TGATCATCTCAGGTGATACTGTCTTTGGGATAATGAAACACCTATTTGTGACAGAATTCCTTGTGGGCTA
 CCCCCACCATCACCAATGGAGATTTCAATTAGCACCAACAGAGAGAATTTTCACTATGGATCAGTGGTGACC
 TACCGCTGCAATCCTGGAAGCGGAGGAGAGAAAGGTGTTTGTAGCTTGTGGGTGAGCCCTCCATATACTGCACC
 AGCAATGACGATCAAGTGGGCATCTGGAGCGGCGCCCGCCCTCAGTGCATTATACCTAACAAATGCACGCGCT
 30 CCAAATGTGGAAAATGGAATATTGGTATCTGACAACAGAAGCTTATTTTCTTAAATGAAGTTGTGGAGTTT
 AGGTGTGAGCCTGGCTTTGTCTGATGAAAGGACCCCGCGGTGTGAAGTGCCAGGCGCTGAACAAATGGGAGCCG
 GAGCTACCAAGCTGCTCCAGGGTATGTGAGCCACCTCCAGATGTCTGCTGATGCTGAGCGTACCCAAAGGGAC
 AAGGACAACCTTTTACCTGGGCAGGAAGTGTCTACAGCTGTGAGCCCGGCTACGACCTCAGAGGGGCTGCG
 TCTATGCGCTGCACACCCAGGGAGACTGGAGCCCTGCAGCCCCACATGTGAAGTGAATCCTGTGATGAC
 35 TTCATGGGCCAATCTTAATGGCCGTGTGCTATTTCCAGTAAATCTCCAGCTTGGAGCAAAAGTGGATTTT
 GTTTGTGATGAAGGATTTCAATTAAGGAGCAGCTCTGCTAGTTACTGTGTCTTGGCTGGAATGGAAGCCTT
 TGGAATAGCAGTGTTCAGTGTGTGAACAAATCTTTGTCCAAGTCTCCAGTTATTCCTAATGGGAGACAC
 ACAGGAAAACCTCTGGAAGTCTTTCCCTTTGGGAAAACAGTAAATTACACATGCGGACCCCAAGGACAGA
 GGGACGAGCTTCGACCTCATTGGAGAGAGCACCATCCGCTGCACAAGTGACCTCAAGGGAATGGGGTTTGG
 40 AGCAGCCCTGCCCCCTGCTGTGGAATCTGGGTACTGTCAAGCCCCAGATCATTTTCTGTTTGCCAAGTTG
 AAAACCCAAACCAATGCATCTGACTTTCCATTGGGACATCTTTAAAGTACGAATGCCGTCTGAGTACTAC
 GGGAGGCCATTCTCTATCATGTCTAGATAACCTGGTCTGGTCAAGTCCCAAAGATGTCTGTAAACGTAAA
 TCATGTAAACTCCTCCAGATCCAGTGAATGGCATGGTGCATGTGATCAGAGATCCAGGTTGGATCCAGA
 45 ATCAACTATTCTTGTACTACAGGGCACCAGCTCATTTGGTCACTCATCTGCTGAATGTATCCTCTCGGGCAAT
 GCTGCCCATTTGGAGCACGAAGCCGCCAATTTGTCAACGAATTCCTTGTGGGCTACCCCCACCATCGCCAAT
 GGAGATTTCAATTAGCACCAACAGAGAGAATTTTCACTATGGATCAGTGGTGACCTACCGCTGCAATCCTGGA
 AGCGGAGGGAGAAAGGTGTTTGTAGCTTGTGGGTGAGCCCTCCATATACTGCACCAGCAATGACGATCAAGTG
 50 GGCATCTGGAGCGGCGCCCGCCCTCAGTGCATTATACCTAACAAATGCACGCTCCAAATGTGGAAAATGGA
 ATATTGGTATCTGACAACAGAAGCTTATTTTCTTAAATGAAGTTGTGGAGTTTAGGTCTACGCTGGCTTT
 GTCATGAAAGGACCCCGCGGTGTGAAGTGCCAGGCGCTGAACAAATGGGAGCCGGAGCTTACCAAGCTGCTCC
 AGGGTATGTGAGCCACCTCCAGATGTCTGCTGATGCTGAGCGTACCCAAAGGACAAAGGACAACCTTTTACCCC
 GGGCAGGAAGTGTCTACAGCTGTGAGCCCGGCTATGACCTCAGAGGGGCTGCGTCTATGCGCTGCACACCC
 CAGGGAGACTGGAGCCCTGCAGCCCCACATGTGAAGTGAATCCTGTGATGACTTCATGGGCCAATCTCTT
 55 AATGGCCGTGTGCTATTTCCAGTAAATCTCCAGCTTGGAGCAAAAGTGGATTTTGTGTGATGAAGGATTT
 CAATTAAGGAGCTGTGCTAGTTATTGTGCTTGGCTGGAATGGAAGCCTTTGGAAATGAGATGTTTCCA
 GTGTGTGAACAAATCTTTGTCCAAGTCTCCAGTTATTTCTAATGGGAGACACACAGGAAAACCTCTGGAA
 GTCTTTCCCTTTGGAAAAGCAGTAAATTACACATGCGACCCCAAGGACAGAGGGACGAGCTTCGACCTC

ATTGGAGAGAGCACCATCCGCTGCACAAGTGACCTCAAGGGAATGGGGTTTGGAGCAGCCCTGCCCCTCGC
 TGTGGAATTTCTGGGTCACTGTCAAGCCCCAGATCATTTTCTGTTTGCCAAGTTGAAAACCCAAACCAATGCA
 TCTGACTTTCCCATTGGGACATCTTTAAAGTACGAATGCCGTCTGAGTACTACGGGAGGGCATTCTCTATC
 ACATGCTTAGATAACCTGGTCTGGTCAAGTCCCAAAGATGTCTGTAAACGTAATCATGTAAAACTCCTCCA
 5 GATCCAGTGAATGGCATGGTGCATGTGATCACAGACATCCAGGTTGGATCCAGAATCAACTATTCTTGTACT
 ACAGGGCACCGACTCATTTGGTCACTCATCTGCTGAATGTATCCTCTCAGGCAATACTGCCCATTTGGAGCAG
 AAGCCGCCAATTTGTCAACGAATTCCTTGTGGGTACCCCCAACCATCGCCAATGGAGATTCATTAGCACC
 AACAGAGAGAATTTTCACTATGGATCAGTGGTGACCTACCGCTGCAATCTTGAAGCAGAGGGAGAAAGGTG
 TTTGAGCTTGTGGGTGAGCCCTCCATATACTGCACCAGCAATGACGATCAAGTGGGCATCTGGAGCGGCCCC
 10 GCCCCTCAGTGCATTATACCTAACAAATGCACGCCCTCCAAATGTGGAAAATGGAATATTGGTATCTGACAAAC
 AGAAGCTTATTTTCCTTAAATGAAGTTGTGGAGTTTAGGTGTCAGCCTGGCTTTGTGATGAAAGGACCCCGC
 CGTGTGAAGTGCCAGGCCCTGAACAAATGGGAGCCAGAGTTACCAAGCTGCTCCAGGGTGTGTAGCCCGCT
 CCAGAAATCCTGCATGGTGAGCATACCCCAAGCCATCAGGACAACTTTTACCTGGGCAGGAAGTGTCTAC
 AGCTGTGAGCCTGGCTATGACCTCAGAGGGGTGCGTCTCTGCACTGCACACCCAGGGAGACTGGAGCCCT
 15 GAAGCCCCGAGATGTGAGTGAATCCTGTGATGACTTCTTGGGTCAACTCCTCATGGCCGTGTGCTATTT
 CCACTTAATCTCCAGCTTGGGGCAAAGGTGTCTTTGTCTGTGATGAAGGGTTTCGCTTAAAGGGCAGTTCC
 GTTAGTCATTGTGTCTTGGTTGGAATGAGAAGCCTTTGGAATAACAGTGTTCCTGTGTGTGAACATATCTTT
 TGTCCAAATCCTCCAGCTATCCTTAATGGGAGACACACAGGAACCTCCCTCTGGAGATATTCCCTATGGAAAA
 GAAATATCTTACACATGTGACCCCCACCCAGACAGAGGGATGACCTTCAACCTCATTGGGGAGAGCACCATC
 20 CGCTGCACAAGTGACCTCATGGGAATGGGGTTTGGAGCAGCCCTGCCCCTCGCTGTGAACCTTTCTGTTCTGT
 GCTGGTCACTGTAAACCCAGAGCAGTTTCCATTGGCCAGTCTACGATCCCAATTAATGACTTTGAGTTT
 CCAGTCGGGACATCTTTGAATTATGAATGCCGTCTGGGTATTTTGGGAAAATGTTCTCTATCTCCTGCCTA
 GAAACTTGGTCTGGTCAAGTGTGGAAGACAACGTAGACGAAAATCATGTGGACCTCCACCAGAACCCTTC
 AATGGAATGGTGCAATATAAACACAGATACACAGTTTGGATCAACAGTTAATTATTCTTGTAAATGAAGGGTTT
 25 CGACTCATTGGTTCCCCATCTACTACTTGTCTCGTCTCAGGCAATAATGTCACATGGGATAAGAAGGCACCT
 ATTTGTGAGATCATATCTTGTGAGCCACCTCCAACCATATCCAATGGAGACTTCTACAGCAACAATAGAACA
 TCTTTTACAATGGAACGGTGGTAACTTACCAGTGGCCACACTGGACCAGATGGAGAACAGCTGTTTGTGCTT
 GTGGGAGAACGGTCAATATATTGCACCAGCAAAGATGATCAAGTTGGTGTGGAGCAGCCCTCCCCCTCGG
 TGTATTTCTACTAATAAATGCACAGCTCCAGAAGTTGAAAATGCAATTAGAGTACCAGGAAACAGGAGTTTC
 30 TTTACCCCTCAGTATGAGATCATCAGATTTAGATGTCAGCCCGGGTTTGTGATGGTAGGGTCCCACACTGTGCAG
 TGCCAGACCAATGGCAGATGGGGGCCAAGCTGCCACACTGCTCCAGGGTGTGTGAGCCGCTCCAGAAATC
 CTGCATGGTGAGCATACCTAAGCCATCAGGACAACTTTTACCTGGGCAGGAAGTGTCTACAGCTGTGAG
 CCCAGCTATGACCTCAGAGGGGTGCGTCTCTGCACTGCACGCCCCAGGGAGACTGGAGCCCTGAAGCCCT
 AGATGTACAGTGAATCCTGTGATGACTTCTGGGCCAACTCCCTCATGGCCGTGTGCTACTTCCACTTAAT
 35 CTCCAGCTTGGGGCAAAGGTGTCTTTGTTTGGCATGAAGGGTCCGATTAAAAGGCAGGTCTGCTAGTCAT
 TGTGTCTTGGCTGGAAATGAAAGCCCTTTGGAATAGCAGTGTTCAGTGTGTGAACAAATCTTTTGTCCAAAT
 CCTCCAGCTATCCTTAATGGGAGACACACAGGAACCTCCCTTTGGAGATATTCCTATGGAAAAGAAATATCT
 TACGCATGCGACACCCACCCAGACAGAGGGATGACCTTCAACCTCATTGGGGAGAGCTCCATCCGCTGCACA
 AGTGACCTCAAGGGAATGGGGTTTGGAGCAGCCCTGCCCCTCGCTGTGAACCTTTCTGTTCTGTGCTGCTGC
 40 CCACATCCACCCAAGATCCAAAACGGGCATTACATTGGAGGACACGTATCTCTATATCTTCTGGGATGACA
 ATCAGCTACATTTGTGACCCCGGCTACCTGTTAGTGGGAAAGGGCTTCATTTTCTGTACAGACCAGGGAATC
 TGGAGCCAATTGGATCATTATTGCAAAGAAGTAAATTGTAGCTTCCCACTGTTTATGAATGGAATCTCGAAG
 GAGTTAGAAAATGAAAAAGTATATCACTATGGAGATTATGTGACTTTGAAGTGTGAAGATGGGTATACTCTG
 GAAGGCAGTCCCTGGAGCCAGTGCCAGGCGGATGACAGATGGGACCCTCCTCTGGCCAAATGTACCTCTCGT
 45 ACACATGATGCTCTCATAGTTGGCACTTTATCTGGTACGATCTTCTTTATTTTACTCATCATTTTCTCTCT
 TGGATAATTCTAAAGCACAGAAAAGGCAATAATGCACATGAAAACCTAAAGAAGTGGCTATCCATTACAT
 TCTCAAGGAGGCAGCAGCGTTTCATCCCCGAACCTCTGCAACAAATGAAGAAAATAGCAGGGTCTTCCCTTGA

(SEQ ID NO: 25)

In some embodiments, the CR1 or fragment thereof is soluble CR1 (sCR1).

- 50 The CR1 fragment is preferably capable of acting as a cofactor for the CFI-mediated cleavage of C3b. The skilled person would be readily able to determine such CFI activity using any suitable method known in the art, for example as disclosed herein.

CR1 contains two known binding sites for C3b (see Liu, D. et al. (2009) Immunopharmacology and Immunotoxicology 31: 524–535). In preferred embodiments, the CR1 fragment comprises one or two C3b binding sites.

5 Preferably, the fragment of CR1 is a soluble fragment of CR1, for example generated by removing the transmembrane and cytoplasmic domains from full length CR1 and/or by selecting CR1 truncations comprising or consisting of certain CCPs.

Example CR1 fragments are known in the art (see, for example, WO2019138137). For example, the CR1 fragment may comprise or consist of CCPs 8-10 (e.g. corresponding to amino acids 491 to 684 of SEQ ID NO: 24) and/or CCPs 15-17 (e.g. corresponding to amino acids 941 to 1134 of SEQ ID NO: 24).

In some embodiments, the CR1 fragment comprises CCPs 8-10. In some embodiments, the CR1 fragment comprises CCPs 15-17. In some embodiments, the CR1 fragment comprises CCPs 8-10 and CCPs 15-17.

15 In other embodiments, the nucleotide sequence encoding CR1 encodes an amino acid sequence that has at least 75%, 80%, 85% 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 24. Preferably, wherein the amino acid sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 24.

In other embodiments, the nucleotide sequence encoding CR1 encodes the amino acid sequence SEQ ID NO: 24.

20 In other embodiments, the nucleotide sequence encoding CR1 encodes an amino acid sequence that has at least 75%, 80%, 85% 90%, 95%, 96%, 97%, 98% or 99% identity to positions 491 to 684 of SEQ ID NO: 24. Preferably, wherein the amino acid sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 24.

25 In other embodiments, the nucleotide sequence encoding CR1 encodes the amino acid sequence positions 491 to 684 of SEQ ID NO: 24.

In other embodiments, the nucleotide sequence encoding CR1 encodes an amino acid sequence that has at least 75%, 80%, 85% 90%, 95%, 96%, 97%, 98% or 99% identity to positions 941 to 1134 of SEQ ID NO: 24. Preferably, wherein the amino acid sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 24.

30 In other embodiments, the nucleotide sequence encoding CR1 encodes the amino acid sequence positions 941 to 1134 of SEQ ID NO: 24.

MEMBRANE COFACTOR PROTEIN (MCP)

Membrane Cofactor Protein (MCP), also known as CD46, is a type I membrane protein that functions as a regulatory part of the complement system and acts as a cofactor for CFI.

The extracellular region of MCP contains four short consensus repeats (SCRs).

- 5 In some embodiments, the MCP or fragment thereof is human MCP or a fragment thereof.

An example MCP sequence is:

MEPPGRRECPFPFSWRFPGLLLAAMVLLLYSFSDACEEPTFEAMELIGKPKPYEIGERVDYKCKKGYFYIP
PLATHITICDRNHTWLPVSDDACRETCPIYIRDPLNGQAVPANGTYEFGYQMHFICNEGYYLIGEEILYCELK
GSVAIWSGKPPICEKVLCTPPPKIKNGKHTFSEVEVFYLDVITYSCDPAPGPDPSLIGESTIYCGDNSVW
10 SRAAPECKVVKCRFPVVENGGKQISGFGKKFYKATVMFECDKGFYLDGSDTIVCDNSNWDPPVPKCKLVLP
PSSTKPPALSHSVSTSTTKSPASSASGPRPTYKPPVSNYPGYPKPEEGILDSLVDVWVIAIVIVIAIVGVAV
ICVVPYRYLQRRKKKGYLTDETHREVKFTSL

(SEQ ID NO: 26)

An example nucleotide sequence encoding MCP is:

15 ATGGAGCCTCCCGGCCCGCGAGTGTCCTTTCTCTGCGCTTTCTGCGGTTGCTTCTGGCGGCCATG
GTGTTGCTGCTGTACTCCTTCTCCGATGCCTGTGAGGAGCCACCAACATTTGAAGCTATGGAGCTCATTGGT
AAACCAAACCTACTATGAGATTGGTGAACGAGTAGATTATAAGTGTAAGGATACTTCTATATACCT
CCTCTTGCCACCCATACTATTTGTGATCGGAATCATACATGGCTACCTGTCTCAGATGACGCTGTTATAGA
20 GAAACATGTCCATATATACGGGATCCTTTAAATGGCCAAGCAGTCCCTGCAAATGGGACTTACGAGTTTGGT
TATCAGATGCACTTTATTTGTAATGAGGGTTATTACTTAATTGGTGAAGAAATCTATATTGTGAAGTTAAA
GGATCAGTAGCAATTTGGAGCGGTAAGCCCCCAATATGTGAAAAGGTTTTGTGTACACCACCTCCAAAAATA
AAAAATGGAAAACACACCTTTAGTGAAGTAGAAGTATTTGAGTATCTTGATGCAGTAACCTATAGTTGTGAT
CCTGCACCTGGACCAGATCCATTTTCACTTATTGGAGAGAGCAGATTTATTGTGGTGACAATTCAGTGTGG
25 AGTCGTGCTGCTCCAGAGTGTAAGTGGTCAATGTGATTTCCAGTAGTCGAAAATGGAAAACAGATATCA
GGATTTGGAAAAAATTTTACTACAAAGCAACAGTTATGTTTGAATGCGATAAGGGTTTTTACCTCGATGGC
AGCGACACAATTTGTCTGTGACAGTAACAGTACTTGGGATCCCCCAGTTCCAAAGTGTCTTAAAGTGCTGCCT
CCATCTAGTACAAAACCTCCAGCTTTGAGTCATTCAAGTGTGACTTCTTCCACTACAAAATCTCCAGCGTCC
AGTGCCCTCAGGTCCTAGGCCTACTTACAAGCCTCCAGTCTCAAATTATCCAGGATATCCTAAACCTGAGGAA
30 GGAATACTTGACAGTTTGGATGTTTGGGTGATTGCTGTGATTGTTATTGCCATAGTTGTTGGAGTTGCAGTA
ATTTGTGTTGTCCCGTACAGATATCTTCAAAGGAGGAAGAAGAAAGGCACATACCTAACTGATGAGACCCAC
AGAGAAGTAAAATTTACTTCTCTCTGA

(SEQ ID NO: 27)

In some embodiments, the MCP or fragment thereof is soluble MCP.

- 35 The MCP fragment is preferably capable of acting as a cofactor for the CFI-mediated cleavage of C3b. The skilled person would be readily able to determine such CFI activity using any suitable method known in the art, for example as disclosed herein.

Preferably, the fragment of MCP is a soluble fragment of MCP, for example generated by removing the transmembrane domains from full length MCP and/or by selecting MCP truncations comprising or consisting of certain SCRs.

In some embodiments, the MCP fragment comprises SCRs 2 and 3. In some embodiments, the MCP fragment comprises SCRs 2, 3 and 4.

In other embodiments, the nucleotide sequence encoding MCP encodes an amino acid sequence that has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 26. Preferably, wherein the amino acid sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 26.

In other embodiments, the nucleotide sequence encoding MCP encodes the amino acid sequence SEQ ID NO: 26.

LINKERS

In preferred embodiments, the nucleotide sequence encoding (i) is upstream of the nucleotide sequence encoding (ii). In other embodiments, the nucleotide sequence encoding (ii) is upstream of the nucleotide sequence encoding (i).

In some embodiments, the nucleotide sequences encoding the (i) and (ii) are operably linked by a linker. In some embodiments, the linker comprises a self-cleaving 2A peptide sequence, such as a sequence comprising or that is defined by a Furin cleavage site, GSG, 11a1D and an F2A sequence.

In some embodiments, the linker is SEQ ID NO: 17.

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CGAAGGAAACGAGGAAGCGGAGAAGCCAGACACAAACAGAAAATTGTGGCACCGGTGAAACAGACTTTGAAT
TTTGACCTTCTCAAGTTGGCGGGAGACGTCGAGTCCAACCTGGGCCC
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(SEQ ID NO: 17)

In other embodiments, the linker has at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 17. Preferably, the linker substantially retains a functional activity of SEQ ID NO: 17.

By “operably linked”, it is to be understood that the individual components are linked together in a manner which enables them to carry out their function substantially unhindered.

PRODUCT

The product of the invention may, for example, be a composition (e.g. a pharmaceutical composition) comprising (i) a Complement Factor I (CFI) cofactor; and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor, in admixture. Alternatively, the product may, for example, be a kit comprising preparations of (i) a Complement Factor I (CFI) cofactor; and (ii) Complement Factor I (CFI), or nucleotide sequences encoding

therefor, and, optionally, instructions for the simultaneous, sequential or separate administration of the preparations to a subject in need thereof.

The product of the invention may, for example, be a composition (e.g. a pharmaceutical composition) comprising (i) Complement Factor H Like Protein 1 (FHL1) or Complement
5 Factor H (CFH); and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor, in admixture. Alternatively, the product may, for example, be a kit comprising preparations of (i) Complement Factor H Like Protein 1 (FHL1) or Complement Factor H (CFH); and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor, and, optionally, instructions for the simultaneous, sequential or separate administration of the
10 preparations to a subject in need thereof.

PROTEIN TRANSDUCTION

As an alternative to the delivery of polynucleotides to cells, the products and agents of the invention may be delivered to cells by protein transduction.

Protein transduction may be via vector delivery (Cai, Y. et al. (2014) Elife 3: e01911;
15 Maetzig, T. et al. (2012) Curr. Gene Ther. 12: 389-409). Vector delivery involves the engineering of viral particles (e.g. lentiviral particles) to comprise the proteins to be delivered to a cell. Accordingly, when the engineered viral particles enter a cell as part of their natural life cycle, the proteins comprised in the particles are carried into the cell.

Protein transduction may be via protein delivery (Gaj, T. et al. (2012) Nat. Methods 9: 805-
20 7). Protein delivery may be achieved, for example, by utilising a vehicle (e.g. liposomes) or even by administering the protein itself directly to a cell.

POLYNUCLEOTIDE

Polynucleotides of the invention may comprise DNA or RNA, preferably DNA. They may be single-stranded or double-stranded. It will be understood by a skilled person that numerous
25 different polynucleotides can encode the same polypeptide as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides of the invention to reflect the codon usage of any particular host organism in which the polypeptides of the invention are to be expressed.

30 The nucleotide sequences of the invention disclosed herein may comprise or lack stop codons at their 3' end, for example depending on their position in a bicistronic vector. Thus,

the present disclosure encompasses the SEQ ID NOs disclosed herein with the stop codons present or absent.

The polynucleotides may be modified by any method available in the art. Such modifications may be carried out in order to enhance the in vivo activity or lifespan of the polynucleotides of the invention.

Polynucleotides such as DNA polynucleotides may be produced recombinantly, synthetically or by any means available to those of skill in the art. They may also be cloned by standard techniques.

Longer polynucleotides will generally be produced using recombinant means, for example using polymerase chain reaction (PCR) cloning techniques. This will involve making a pair of primers (e.g. of about 15 to 30 nucleotides) flanking the target sequence which it is desired to clone, bringing the primers into contact with mRNA or cDNA obtained from an animal or human cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture with an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable vector.

In some embodiments, the polynucleotide comprises or consists of the nucleotide sequence of SEQ ID NO: 22, or a nucleotide sequence that has at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereto.

CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCCGCCGGCCTCAGTGAGC
GAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCTTGTAGTTAATGATTAACCCGCCA
TGCTACTTATCTACGTAGCCATGCTCTAGGTACCGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGC
CTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG
GGACTTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATC
ATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGA
CCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATTGGTGATGCGGTTTT
GGCAGTACATCAATGGGCGTGGATAGCGGTTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATGACGTCA
ATGGGAGTTTGTGGTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCATGACGC
AAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCT
GGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGACTAGTGCCACCATGCGCCTCCTGGCCAA
GATCATCTGCCTCATGCTGTGGGCCATCTGCGTGGCTGAGGACTGCAATGAGCTGCCGCCAGGAGGAACAC
AGAGATCCTGACAGGGAGCTGGTCTGACCAGACCTACCCTGAGGGCACCCAGGCGATCTACAAGTGCCGGCC
GGGCTACAGGAGCCTGGGGAACATCATCATGGTGTGTAGAAAGGGCGAATGGGTGGCCCTCAACCCCTGAG
GAAGTGCCAGAAGCGGCCCTGTGGCCACCCCGGGGACACACCCTTCGGGACCTTCACCTGACCGGCGGCAA
TGTGTTTGTGAGTACGGCTGAAGGCTGTCTACACATGCAACGAGGGGTACCAGCTGCTGGGCGAGATTAAC
CCGGGAGTGTGACACCGATGGGTGGACCAACGACATTCCTCATCTGTGAGGTGGTCAAGTGTCTCCCGTGAC
AGCCCCAGAAAATGGCAAAATCGTGAGCAGCGCATGGAGCCTGACCGCAATATCACTTTGGGCAGGCCGT
GAGGTTTGTGTGCAACTCGGGCTACAAAATTGAAGGTGATGAGGAGATGCACTGCAGCGATGATGGCTTCTG
GTCCAAGGAGAAGCCCAATGTGTGGAGATCTCTGCAAGTCTCCCGACGTGATCAACGGCAGCCCAATCAG
CCAGAAGATTATTTACAAAGAGAACGAGCGCTTCCAGTACAAGTGAACATGGGCTATGAGTATTAGAGAG

GGGAGATGCCGTCTGCACTGAGAGCGGCTGGAGACCACTGCCTAGCTGCGAGGAAAAGAGTTGTGACAACCC
 TTACATCCCAAATGGCGACTACTCCCCTCTGCGGATCAAACACCGGACCGGGGATGAAATCACCTATCAGTG
 CCGCAATGGATTCTACCCGGCCACCCGCGGCAACACCGCCAAATGCACCAGCACAGGCTGGATCCCCGGCCC
 CCGCTGTACGCTGAAGCCTTGCGACTATCCAGACATCAAGCACGGAGGCCCTGTACCACGAAAACATGCGGCG
 5 GCCTTATTTCCCTGTGGCAGTGGGGAAGTACTACAGCTACTACTGCGACGAGCACTTCGAGACCCCCCTCTGG
 CTCTACTGGGACCACATCCACTGCACACAGGACGGCTGGTCTCCAGCTGTGCCCTGCCGTAGGAAATGCTA
 CTTCCCTACCTGGAGAACGGATACAACCAGAACTATGGCCGCAAGTTCGTGCAGGGCAAGAGCATCGATGT
 GGCCTGCCACCCTGGCTACGCCCTGCCCAAGGCCAGACAACCTGTGACCTGCATGGAGAATGGTTGGAGCCC
 CACCCCGCGCTGCATCCGGGTGTCTTCACGCTCCGAAGGAAACGAGGAAGCGGAGAAGCCAGACACAAACA
 10 GAAAATTGTGGCACCCTGAAACAGACTTTGAATTTTGACCTTCTCAAGTTGGCGGGAGACGTGAGTCCAA
 CCCTGGGCCCATGAACTGCTGCATGTCTTCTCTCTCTCTCTGTGCTTCCACCTCCGTTTCTGTAAAGTCAC
 CTACACTAGCCAGGAGGATCTGGTGGAGAAGAAAATGCCTGGCCAAGAAGTATACCCACCTGAGCTGCCACAA
 AGTGTCTGCCAGCCCTGGCAACGCTGCATTGAAGGTACTTGTGTGCAAGCTGCCCTACCAGTGCCCCAA
 GAACGGCACGGCCGTGTGTGCCACCAACAGGAGGAGCTTCCCCACCTACTGCCAGCAGAAGAGCCTGGAATG
 15 CCTCCACCTGGCACCAAGTTTCTGAACAACGGGACCTGCACAGCCGAGGGGAAATTCAGGCTCTCCCTCAA
 GCACGGCAATACAGACTCCGAGGGCATTGTGGAAGTGAAGCTGGTGGACCAGGACAAGACCATTGTTCACTG
 CAAAAGCAGCTGGTCCATGCGGGAGGCCAATGTGCGCTGCCCTGGACCTGGGCTTCCAGCAGGGCGCTGATAC
 ACAGCGCCGCTTTAAACTCAGTGACCTCAGCATCAACAGCACTGAGTGTCTGCACGTGCAGTGCCTGGCGGCT
 GGAGACCAGCCTGGCTGAGTGACCTTACCAAGCGCAGGACCATTGGGCTACCAGGATTTTGCAGATGTGGT
 20 CTGCTACACCCAGAAGGCAGACAGCCCCATGGATGACTTCTTCCAGTGTGTCAATGGCAAGTACATTTCCCA
 GATGAAGGCTTGTGACGGGATCAATGATTGCGGGGATCAGAGCGATGAGCTGTGCTGCAAGGCCTGCCAAGG
 GAAGGGCTTTCACTGTAAGTCTGGGGTGTGCATCCCTTCTCAGTATCAGTGCAACGGAGAGGTGGACTGCAT
 CACTGGGGAGGACGAGGTGGGCTGTGCTGGCTTGCCTCTGTGGCCAGGAGGAGACAGAGATCCTCACAGC
 TGACATGGATGCAGAGCGGCGGGCATCAAGAGTCTGCTCCCAAAGCTCTCCTGCGGCGTTAAGAATCGCAT
 25 GCACATCCGGAGGAAGCGGATCGTTGGAGGCAACGGGCTCAGCTGGGGGACTTGCCGTGGCAGGTGGCCAT
 CAAAGATGCCCTCCGGAATCACCTGTGGTGGCATCTACATCGGCGGCTGCTGGATCCTGACCGCCGCCACTG
 CCTTCCGGCCAGCAAGACTCACCGCTACCAGATCTGGACCACCGTGGTGGATTGGATTACCCCCGACCTGAA
 GAGGATTTGATTGAGTATGTGACCGCATCATCTTCCATGAAAACATAATGCCGGGACGTATCAGAACGA
 CATCGCCCTCATCGAGATGAAGAAGGATGGGAACAAGAAGGACTGTGAGCTGCCTCGCTCCATCCCCGCCTG
 30 TGTACCATGGTCTCCGTACCTGTTCCAGCCAAATGACACATGCATCGTGAGCGGCTGGGCGCGCGAGAAAGA
 CAACGAGAGGGTCTTCTCCCTGCAGTGGGGTGAAGTCAAGCTGATCAGCAACTGCTCCAAGTTCTACGGCAA
 CCGCTTCTATGAGAAGGAGATGGAGTGGCCGGCACCTATGACGGCAGCATTGACGCGTGAAGGGAGACAG
 TGGGGGCCCCCTGGTCTGCATGGACGCCAACAAATGTGACCTACGTGTGGGGAGTTGTGTCTGGGGCGAGAA
 CTGTGGCAAGCCTGAGTTCCCGGGCGTGTACACAAAGGTGGCAAACTATTTGACTGGATCTCCTATCACGT
 35 TGGCAGGCCCTTCATTTACAGTACAACGTATAACTCGAGAATCAACCTCTGGATTACAAAATTTGTGAAAG
 ATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCCTTTGTATCA
 TGCTATTGCTTCCCGTATGGCTTTTCAATTTCTCTCTCTTGTATAAATCCTGGTTAGTTCTTGCCACGGCGGA
 ACTCATCGCCGCTGCTTGGCCGCTGCTGGACAGGGGCTCGGCTGTGGGCACTGACAAATCCGTGGTGTG
 CTTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCTTCTTGAACCTGGAAGGTGCCACTTCC
 40 ACTGTCCTTTCTAATAAAATGAGGAAATGTCATCGCATTGTCTGAGTAGGTGTATTCTATTCTGGGGGGT
 GGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAAATAGCAGGCATGCTGGGGATGCGGTGGGCTCT
 ATGGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCGACTAGAGCATGGCTACGTAGATAAGTAGCATGGCGG
 GTTAATCATTAATACTAAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCAC
 TGAGGCCGGGGGACCAAAGGTGCGCCGACGCCCCGGCTTTGCCCGGGCGGCTCAGTGAGCGAGCG
 45 (SEQ ID NO: 22)

In some embodiments, the polynucleotide comprises or consists of the nucleotide sequence of SEQ ID NO: 23, or a nucleotide sequence that has at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereto.

50 CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTGGGCGACCTTTGGTGCGCCGGCCCTCAGTGAGC
 GAGCGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTTCCTGGCGCGCCGGAGTTCCGCGTTAC
 ATAACCTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATGACGTCAATAATGACGTA
 TGTCCCATAGTAACGCCAATAGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAATGCCCA
 55 CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
 CTGGCATTATGCCAGTACATGACCTTATGGGACTTCTCTACTTGGCAGTACATCTACGTATTAGTCATCGC

TATTACCATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTC
AAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTGGTGGCACCACAAATCAACGGGACTTTCCAAAATGTCTG
TAACAACCTCCGCCCCATTGACGCAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCG
TTTAGTGAAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGACTAGT
5 GCCACCATGCGCCTCCTGGCCAAGATCATCTGCCTCATGCTGTGGGCCATCTGCGTGGCTGAGGACTGCAAT
GAGCTGCCGCCCCAGGAGGAACACAGAGATCCTGACAGGGAGCTGGTCTGACCAGACCTACCCTGAGGGCACC
CAGGCGATCTACAAGTGCCGCGCGGCTACAGGAGCCTGGGGAACATCATCATGGTGTGTAGAAAGGGCGAA
TGGGTGGCCCTCAACCCCTGAGGAAGTGCCAGAAGCGGCCCTGTGGCCACCCCGGGGACACACCTTTCGGG
ACCTTCACCCCTGACCGCGGCAATGTGTTTGTAGTACGGCGTGAAGGCTGTCTACACATGCACGAGGGGTAC
10 CAGCTGCTGGGCGAGATTAACCTACCGGGAGTGTGACACCGATGGGTGGACCAACGACATTTCCCATCTGTGAG
GTGGTCAAGTGTCTCCCGTGACAGCCCCAGAAAAATGGCAAAATCGTGAGCAGCGCCATGGAGCCTGACCGC
GAATATCACTTTGGGCAGGCCGTGAGGTTTGTGTGCAACTCGGGCTACAAAATTGAAGGTGATGAGGAGATG
CACTGCAGCGATGATGGCTTCTGGTCCAAGGAGAAGCCCCAAATGTGTGGAGATCTCCTGCAAGTCTCCCGAC
15 GTGATCAACGGCAGCCCAATCAGCCAGAAGATTATTTACAAAGAGAACGAGCGCTTCCAGTACAAGTGTAAAC
ATGGGCTATGAGTATTCAGAGAGGGGAGATGCCCTCTGCACTGAGAGCGGCTGGAGACCACTGCCTAGTGTG
GAGGAAAAGAGTTGTGACAACCTTACATCCCAATGGCGACTACTCCCTCTGCGGATCAAACACCGGACC
GGGGATGAAATCACCTATCAGTGCCGCAATGGATTCTACCGGCCACCCGCGGCAACACCGCCAAATGCACC
AGCACAGGCTGGATCCCCGCCCGCGCTGTACGCTGAAGCCTTGGCACTATCCAGACATCAAGCACGGAGGC
CTGTACCACGAAAACATGCGGCGGCTTATTTCCCTGTGGCAGTGGGGAAGTACTACAGTACTACTGCGAC
20 GAGCACTTCGAGACCCCTCTGGCTCCTACTGGGACCACATCCACTGCACACAGGACGGCTGGTCTCCAGCT
GTGCCCTGCCTGAGGAAATGCTACTTCCCTACTCTGGAGAACGGATACAACCAGAACTATGGCCGCAAGTTC
GTGCAGGCAAGAGCATCGATGTGGCCTGCCACCTGGCTACGCCCTGCCCAAGGCCAGACAACCTGTGACC
TGCATGGAGAATGGTTGGAGCCCCACCCGCGCTGCATCCGGGTGTCTTTCAGCTCCGAAGGAAACGAGGA
AGCGGAGAAGCCAGACACAAACAGAAAAATTGTGGCACCGGTGAAACAGACTTTGAATTTTGACCTTCTCAAG
25 TTGGCGGGAGACGTCGAGTCCAACCTGGGCCCCATGAAACTGCTGCATGTCTTCTCTCTTCTCTGTGCTTC
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30 TGGAAATTCAGCGCTCCCTCAAGCACGGCAATACAGACTCCGAGGGCATTGTGGAAGTGAAGCTGGGAC
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GGCTTCCAGCAGGGCGCTGATACACAGCGCCGCTTTAAACTCAGTGACCTCAGCATCAACAGCACTGAGTGT
CTGCACGTGCACTGCCGGGGCCTGGAGACCAGCCTGGCTGAGTGCACCTTACCAAGCGCAGGACCATGGGC
TACCAGGATTTTGCAGATGTGGTCTGCTACACCCAGAAGGCAGACAGCCCCATGGATGACTTCTTCCAGTGT
35 GTCAATGGCAAGTACATTTCCAGATGAAGGCTTGTGACGGGATCAATGATTGCGGGGATCAGAGCGATGAG
CTCCTGCTGCAAGGCCCTGCCAAGGGAAGGGCTTTCACTGTAAGTCTGGGGTGTGCATCCCTTCTCAGTATCAG
TGCAACGGAGAGGTGGACTGCATCACTGGGAGGACGAGGTGGGCTGTGCTGGCTTCCGCTCTGTGGCCCCAG
GAGGAGACAGAGATCCTCACAGCTGACATGGATGCAGAGCGGGCGCATCAAGAGTCTGTCTCCAAAGCTC
40 TCCTGCGGCGTTAAGAAATCGCATGCACATCCGGAGGAAGCGGATCGTTGGAGGCAACGGGCTCAGCTGGGG
GACTTGCCGTGGCAGGTGGCCATCAAAGATGCCTCCGGAATCACCTGTGGTGGCATCTACATCGGCGGCTGC
TGGATCTTGACCGCGCCCACTGCCTTCGGGGCAGCAAGACTACCGCTACCAGATCTGGACCACTGGTGTG
GATTGGATTACCCCGACCTGAAGAGGATTGTCTATTGAGTATGTGACCGCATCATCTTCCATGAAAACACTAC
AATGCCGGGACGTATCAGAACGACATCGCCCTCATCGAGATGAAGAAGGATGGGAACAAGAAGGACTGTGAG
45 CTGCGCTCGCTCCATCCCGCCTGTGTACCATGGTCTCCGTACCTGTTCCAGCCAAATGACACATGCATCGTG
AGCGGCTGGGGCCGCGAGAAAGACAACGAGAGGGTCTTCTCCCTGCACTGGGGTGAAGTCAAGCTGATCAGC
AACTGCTCCAAGTTCTACGGCAACCGCTTCTATGAGAAGGAGATGGAGTGCGCCGGCACCTATGACGGCAGC
ATTGACGCGTGCAAGGGAGACAGTGGGGGCCCTGGTCTGCATGGACGCCAACAATGTGACCTACGTGTGG
GGAGTTGTCTGCTGGGGCGAGAACTGTGGCAAGCCTGAGTTCCCGGGCGGTACACAAAGGTGGCAAACTAT
50 TTTGACTGGATCTCCTATCACGTTGGCAGGCCCTTCAATTTACAGTACAACGTATAACTCGAGAATCAACCT
CTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACATATGTTGCTCCTTTTACGCTATGTGGATAC
GCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTTCAATTTCTCTCTCTGTATAAATCC
TGGTTAGTTCTTGCCACGGCGGAACTCATCGCCGCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTG
GGCACTGACAATTCCTGGTGTGCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCT
55 TGACCTGGAAGGTGCCACTCCCCTGTCTTTCTAATAAAATGAGGAAATGTCATCGCATGTCTGAGTA
GGTGTCTATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGC
ATGCTGGGGATGCGGTGGGCTCTATGGGCGGCCGAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCT
GCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAGGTGCGCCGACGCCCCGGGCTTTGCCCCGGCGGCGCTC
AGTGAGCGAGCG

(SEQ ID NO: 23)

STRUCTURE OF THE EYE

The medicaments disclosed herein may be delivered to a mammalian, preferably human eye in relation to the treatment or prevention of an eye disease, such as age-related macular degeneration (AMD).

- 5 The person skilled in the treatment of diseases of the eye will have a detailed and thorough understanding of the structure of the eye. However, the following structures of particular relevance to the invention are described.

Retina

- 10 The retina is the multi-layered membrane, which lines the inner posterior chamber of the eye and senses an image of the visual world which is communicated to the brain via the optic nerve. In order from the inside to the outside of the eye, the retina comprises the layers of the neurosensory retina and retinal pigment epithelium, with the choroid lying outside the retinal pigment epithelium.

Neurosensory retina and photoreceptor cells

- 15 The neurosensory retina harbours the photoreceptor cells that directly sense light. It comprises the following layers: internal limiting membrane (ILM); nerve fibre layer; ganglion cell layer; inner plexiform layer; inner nuclear layer; outer plexiform layer; outer nuclear layer (nuclei of the photoreceptors); external limiting membrane (ELM); and photoreceptors (inner and outer segments of the rods and cones).
- 20 The skilled person will have a detailed understanding of photoreceptor cells. Briefly, photoreceptor cells are specialised neurons located in the retina that convert light into biological signals. Photoreceptor cells comprise rod and cone cells, which are distributed differently across the retina.

- 25 Rod cells are distributed mainly across the outer parts of the retina. They are highly sensitive and provide for vision at low light levels. There are on average about 125 million rod cells in a normal human retina.

- Cone cells are found across the retina, but are particularly highly concentrated in the fovea, a pit in the neurosensory retina that is responsible for central high resolution vision. Cone cells are less sensitive than rod cells. There are on average about 6-7 million cone cells in a normal human retina.
- 30

Retinal pigment epithelium

The retinal pigment epithelium (RPE) is a pigmented layer of cells located immediately to the outside of the neurosensory retina. The RPE performs a number of functions, including transport of nutrients and other substances to the photoreceptor cells, and absorption of scattered light to improve vision.

5 Choroid

The choroid is the vascular layer situated between the RPE and the outer sclera of the eye. The vasculature of the choroid enables provision of oxygen and nutrients to the retina.

AGE-RELATED MACULAR DEGENERATION (AMD)

10 The clinical progression of age-related macular degeneration (AMD) is characterised in stages according to changes in the macula. The hallmark of early AMD is the appearance of drusen, which are accumulations of extracellular debris underneath the retina and appear as yellow spots in the retina during clinical examination and on fundus photographs. Drusen are categorised by size as small ($<63\ \mu\text{m}$), medium ($63\text{--}124\ \mu\text{m}$) and large ($>124\ \mu\text{m}$). They are also considered as hard or soft depending on the appearance of their margins on
15 ophthalmological examination. While hard drusen have clearly defined margins, soft drusen have less defined, fluid margins. The Age-related Eye Disease Study (AREDS) fundus photographic severity scale is one of the main classification systems used for this condition.

AMD is classified into “dry” and “wet” (exudative or neovascular) forms. Dry AMD is more common than wet AMD, but the dry form can progress to the wet form, and the two occur
20 simultaneously in a significant number of cases. Dry AMD is typically characterised by progressive apoptosis of cells in the RPE layer, overlying photoreceptor cells, and frequently also the underlying cells in the choroidal capillary layer. Confluent areas of RPE cell death accompanied by overlying photoreceptor atrophy are referred to as geographic atrophy (GA). Patients with this form of AMD experience a slow and progressive deterioration in
25 central vision.

Wet AMD is characterised by bleeding and/or leakage of fluid from abnormal vessels that have grown from the choroidal vessels (choriocapillaris) beneath the RPE and the macula, which can be responsible for sudden and disabling loss of vision. It has been estimated that much of the vision loss that patients experience is due to such choroidal neovascularisation
30 (CNV) and its secondary complications.

The treatment or prevention of AMD described herein may reduce or prevent the appearance of an AMD phenotype described above. Preferably, the treatment of AMD enables maintenance or improvement in visual function.

5 In some embodiments, the treatment or prevention of AMD results in a prevention of or reduction in the formation of geographic atrophy. In other embodiments, the treatment or prevention of AMD results in slowing the progression of geographic atrophy. For example, it results in an at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% reduction in the increase in GA area over the 12 months following administration to a treated eye of a subject, relative to an untreated eye over the same period. In other embodiments, the
10 treatment or prevention of AMD results in the treatment of geographic atrophy, for example a reduction in the amount of geographic atrophy.

In some embodiments, the treatment or prevention of AMD results in a prevention of or reduction in the formation of drusen. In other embodiments, the treatment or prevention of AMD results in a reduction in existing drusen, for example a reduction in the size and/or
15 number of existing drusen.

In some embodiments, the treatment or prevention of AMD results in a prevention of or reduction in complement deposition. In other embodiments, the treatment or prevention of AMD results in a reduction in existing complement deposition.

20 In some embodiments, the treatment or prevention of AMD results in an improvement in or restoration of vision or visual acuity. In other embodiments, the treatment or prevention of AMD mitigates the loss of vision or visual acuity.

In some embodiments, the treatment or prevention of AMD results in an improvement in or restoration of reading speed in a subject. In other embodiments, the treatment or prevention of AMD mitigates the reduction in reading speed in a subject.

25 In some embodiments, the treatment or prevention of AMD results in a reduction or prevention of loss of photoreceptors and/or the retinal pigment epithelium (RPE).

DIABETIC RETINOPATHY

Diabetic retinopathy is a condition characterised by damage to the blood vessels of the retina, which is caused by the high blood sugar levels associated with diabetes. If left
30 untreated, diabetic retinopathy can cause blindness.

Although subjects with mild diabetic retinopathy may have good vision, certain types of diabetic retinopathy, namely diabetic macular oedema (DMO) and proliferative diabetic retinopathy (PDR) may threaten the sight of the subject.

5 Diabetic macular oedema is characterised by the leakage of fluid from the damaged blood vessels in the back of the eye. The leaked fluid accumulates in the macula, which leads to swelling and blurred vision. This can eventually give rise to poor central vision and an inability to read or drive. Side vision usually remains normal.

10 Proliferative diabetic retinopathy is characterised by the closure of retinal blood vessels, leading to the growth of abnormal, fragile blood vessels on the surface of the retina. This may result in permanent loss of vision due to bleeding into the eye, scarring and retinal detachment. Non-proliferative retinopathy is the early stage of diabetic retinopathy which may lead to proliferative retinopathy if left untreated. Therefore treatments are contemplated to all stages and types of diabetic retinopathy.

VECTORS

15 A vector is a tool that allows or facilitates the transfer of an entity from one environment to another.

Adeno-associated viral (AAV) vectors

In one aspect, the invention provides an AAV vector comprising a polynucleotide of the invention.

20 Preferably, the AAV vector is in the form of an AAV vector particle.

Methods of preparing and modifying viral vectors and viral vector particles, such as those derived from AAV, are well known in the art.

The AAV vector may comprise an AAV genome or a fragment or derivative thereof.

25 AAV is known to be capable of packaging genomes up to 5.2 kb in size (Dong, J.-Y. et al. (1996) Human Gene Therapy 7: 2101-2112).

An AAV genome is a polynucleotide sequence, which may encode functions needed for production of an AAV particle. These functions include those operating in the replication and packaging cycle of AAV in a host cell, including encapsidation of the AAV genome into an AAV particle. Naturally occurring AAVs are replication-deficient and rely on the provision of

helper functions in trans for completion of a replication and packaging cycle. Accordingly, the AAV genome of the AAV vector of the invention is typically replication-deficient.

The AAV genome may be in single-stranded form, either positive or negative-sense, or alternatively in double-stranded form. The use of a double-stranded form allows bypass of the DNA replication step in the target cell and so can accelerate transgene expression.

The AAV genome may be from any naturally derived serotype, isolate or clade of AAV. Thus, the AAV genome may be the full genome of a naturally occurring AAV. As is known to the skilled person, AAVs occurring in nature may be classified according to various biological systems.

Commonly, AAVs are referred to in terms of their serotype. A serotype corresponds to a variant subspecies of AAV which, owing to its profile of expression of capsid surface antigens, has a distinctive reactivity which can be used to distinguish it from other variant subspecies. Typically, a virus having a particular AAV serotype does not efficiently cross-react with neutralising antibodies specific for any other AAV serotype.

AAV serotypes include AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 and AAV11, and also recombinant serotypes, such as Rec2 and Rec3, recently identified from primate brain. Any of these AAV serotypes may be used in the invention.

In some embodiments, the AAV vector particle is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rec2 or Rec3 AAV vector particle.

In some embodiments, the AAV may be an AAV1, AAV2, AAV5, AAV7 or AAV8 serotype.

In some embodiments, the AAV may be an AAV2 or AAV8 serotype.

In some embodiments, the AAV may be an AAV2 serotype. In other embodiments, the AAV may be an AAV8 serotype.

The capsid protein may be a mutant capsid protein such as disclosed in WO 2008/124724, which is hereby incorporated by reference.

In some embodiments, the AAV vector comprises an AAV8 capsid with an Y733F mutation.

Reviews of AAV serotypes may be found in Choi et al. (2005) Curr. Gene Ther. 5: 299-310 and Wu et al. (2006) Molecular Therapy 14: 316-27. The sequences of AAV genomes or of elements of AAV genomes including ITR sequences, rep or cap genes for use in the invention may be derived from the following accession numbers for AAV whole genome

sequences: Adeno-associated virus 1 NC_002077, AF063497; Adeno-associated virus 2 NC_001401; Adeno-associated virus 3 NC_001729; Adeno-associated virus 3B NC_001863; Adeno-associated virus 4 NC_001829; Adeno-associated virus 5 Y18065, AF085716; Adeno-associated virus 6 NC_001862; Avian AAV ATCC VR-865 AY186198, 5 AY629583, NC_004828; Avian AAV strain DA-1 NC_006263, AY629583; Bovine AAV NC_005889, AY388617.

AAV may also be referred to in terms of clades or clones. This refers to the phylogenetic relationship of naturally derived AAVs, and typically to a phylogenetic group of AAVs which can be traced back to a common ancestor, and includes all descendants thereof. 10 Additionally, AAVs may be referred to in terms of a specific isolate, i.e. a genetic isolate of a specific AAV found in nature. The term genetic isolate describes a population of AAVs which has undergone limited genetic mixing with other naturally occurring AAVs, thereby defining a recognisably distinct population at a genetic level.

The skilled person can select an appropriate serotype, clade, clone or isolate of AAV for use 15 in the invention on the basis of their common general knowledge. For instance, the AAV5 capsid has been shown to transduce primate cone photoreceptors efficiently as evidenced by the successful correction of an inherited colour vision defect (Mancuso et al. (2009) Nature 461: 784-7).

The AAV serotype determines the tissue specificity of infection (or tropism) of an AAV. 20 Accordingly, preferred AAV serotypes for use in AAVs administered to patients in accordance with the invention are those which have natural tropism for or a high efficiency of infection of target cells within the eye. In some embodiments, AAV serotypes for use in the invention are those which transduce cells of the neurosensory retina, retinal pigment epithelium and/or choroid.

25 Typically, the AAV genome of a naturally derived serotype, isolate or clade of AAV comprises at least one inverted terminal repeat sequence (ITR). An ITR sequence acts in cis to provide a functional origin of replication and allows for integration and excision of the vector from the genome of a cell. In preferred embodiments, one or more ITR sequences flank the nucleotide sequences encoding the Complement Factor I, and/or Complement 30 Factor H or FHL1. The AAV genome typically also comprises packaging genes, such as rep and/or cap genes which encode packaging functions for an AAV particle. The rep gene encodes one or more of the proteins Rep78, Rep68, Rep52 and Rep40 or variants thereof. The cap gene encodes one or more capsid proteins such as VP1, VP2 and VP3 or variants

thereof. These proteins make up the capsid of an AAV particle. Capsid variants are discussed below.

A promoter will be operably linked to each of the packaging genes. Specific examples of such promoters include the p5, p19 and p40 promoters (Laughlin et al. (1979) Proc. Natl. Acad. Sci. USA 76: 5567-5571). For example, the p5 and p19 promoters are generally used to express the rep gene, while the p40 promoter is generally used to express the cap gene.

As discussed above, the AAV genome used in the AAV vector of the invention may therefore be the full genome of a naturally occurring AAV. For example, a vector comprising a full AAV genome may be used to prepare an AAV vector or vector particle in vitro. However, while such a vector may in principle be administered to patients, this will rarely be done in practice. Preferably, the AAV genome will be derivatised for the purpose of administration to patients. Such derivatisation is standard in the art and the invention encompasses the use of any known derivative of an AAV genome, and derivatives which could be generated by applying techniques known in the art. Derivatisation of the AAV genome and of the AAV capsid are reviewed in Coura and Nardi (2007) Virology Journal 4: 99, and in Choi et al. and Wu et al., referenced above.

Derivatives of an AAV genome include any truncated or modified forms of an AAV genome which allow for expression of a transgene from an AAV vector of the invention in vivo. Typically, it is possible to truncate the AAV genome significantly to include minimal viral sequence yet retain the above function. This is preferred for safety reasons to reduce the risk of recombination of the vector with wild-type virus, and also to avoid triggering a cellular immune response by the presence of viral gene proteins in the target cell.

Typically, a derivative will include at least one inverted terminal repeat sequence (ITR), preferably more than one ITR, such as two ITRs or more. One or more of the ITRs may be derived from AAV genomes having different serotypes, or may be a chimeric or mutant ITR. A preferred mutant ITR is one having a deletion of a trs (terminal resolution site). This deletion allows for continued replication of the genome to generate a single-stranded genome which contains both coding and complementary sequences, i.e. a self-complementary AAV genome. This allows for bypass of DNA replication in the target cell, and so enables accelerated transgene expression.

The one or more ITRs will preferably flank the nucleotide sequence encoding the Complement Factor I, and/or CFI cofactor (e.g. Complement Factor H or FHL1) at either end. The inclusion of one or more ITRs is preferred to aid concatamer formation of the vector of the invention in the nucleus of a host cell, for example following the conversion of

single-stranded vector DNA into double-stranded DNA by the action of host cell DNA polymerases. The formation of such episomal concatamers protects the vector construct during the life of the host cell, thereby allowing for prolonged expression of the transgene in vivo.

- 5 In preferred embodiments, ITR elements will be the only sequences retained from the native AAV genome in the derivative. Thus, a derivative will preferably not include the rep and/or cap genes of the native genome and any other sequences of the native genome. This is preferred for the reasons described above, and also to reduce the possibility of integration of the vector into the host cell genome. Additionally, reducing the size of the AAV genome
10 allows for increased flexibility in incorporating other sequence elements (such as regulatory elements) within the vector in addition to the transgene.

The following portions could therefore be removed in a derivative of the invention: one inverted terminal repeat (ITR) sequence, the replication (rep) and capsid (cap) genes. However, in some embodiments, derivatives may additionally include one or more rep and/or
15 cap genes or other viral sequences of an AAV genome. Naturally occurring AAV integrates with a high frequency at a specific site on human chromosome 19, and shows a negligible frequency of random integration, such that retention of an integrative capacity in the vector may be tolerated in a therapeutic setting.

Where a derivative comprises capsid proteins i.e. VP1, VP2 and/or VP3, the derivative may
20 be a chimeric, shuffled or capsid-modified derivative of one or more naturally occurring AAVs. In particular, the invention encompasses the provision of capsid protein sequences from different serotypes, clades, clones, or isolates of AAV within the same vector (i.e. a pseudotyped vector).

Chimeric, shuffled or capsid-modified derivatives will be typically selected to provide one or
25 more desired functionalities for the AAV vector. Thus, these derivatives may display increased efficiency of gene delivery, decreased immunogenicity (humoral or cellular), an altered tropism range and/or improved targeting of a particular cell type compared to an AAV vector comprising a naturally occurring AAV genome, such as that of AAV2. Increased efficiency of gene delivery may be effected by improved receptor or co-receptor binding at
30 the cell surface, improved internalisation, improved trafficking within the cell and into the nucleus, improved uncoating of the viral particle and improved conversion of a single-stranded genome to double-stranded form. Increased efficiency may also relate to an altered tropism range or targeting of a specific cell population, such that the vector dose is not diluted by administration to tissues where it is not needed.

Chimeric capsid proteins include those generated by recombination between two or more capsid coding sequences of naturally occurring AAV serotypes. This may be performed for example by a marker rescue approach in which non-infectious capsid sequences of one serotype are co-transfected with capsid sequences of a different serotype, and directed
5 selection is used to select for capsid sequences having desired properties. The capsid sequences of the different serotypes can be altered by homologous recombination within the cell to produce novel chimeric capsid proteins.

Chimeric capsid proteins also include those generated by engineering of capsid protein sequences to transfer specific capsid protein domains, surface loops or specific amino acid
10 residues between two or more capsid proteins, for example between two or more capsid proteins of different serotypes.

Shuffled or chimeric capsid proteins may also be generated by DNA shuffling or by error-prone PCR. Hybrid AAV capsid genes can be created by randomly fragmenting the sequences of related AAV genes e.g. those encoding capsid proteins of multiple different
15 serotypes and then subsequently reassembling the fragments in a self-priming polymerase reaction, which may also cause crossovers in regions of sequence homology. A library of hybrid AAV genes created in this way by shuffling the capsid genes of several serotypes can be screened to identify viral clones having a desired functionality. Similarly, error prone PCR may be used to randomly mutate AAV capsid genes to create a diverse library of variants
20 which may then be selected for a desired property.

The sequences of the capsid genes may also be genetically modified to introduce specific deletions, substitutions or insertions with respect to the native wild-type sequence. In particular, capsid genes may be modified by the insertion of a sequence of an unrelated protein or peptide within an open reading frame of a capsid coding sequence, or at the N-
25 and/or C-terminus of a capsid coding sequence.

The unrelated protein or peptide may advantageously be one which acts as a ligand for a particular cell type, thereby conferring improved binding to a target cell or improving the specificity of targeting of the vector to a particular cell population. An example might include the use of RGD peptide to block uptake in the retinal pigment epithelium and thereby
30 enhance transduction of surrounding retinal tissues (Cronin et al. (2008) ARVO Abstract: D1048). The unrelated protein may also be one which assists purification of the viral particle as part of the production process, i.e. an epitope or affinity tag. The site of insertion will typically be selected so as not to interfere with other functions of the viral particle e.g. internalisation, trafficking of the viral particle. The skilled person can identify suitable sites for

insertion based on their common general knowledge. Particular sites are disclosed in Choi et al., referenced above.

The invention additionally encompasses the provision of sequences of an AAV genome in a different order and configuration to that of a native AAV genome. The invention also
5 encompasses the replacement of one or more AAV sequences or genes with sequences from another virus or with chimeric genes composed of sequences from more than one virus. Such chimeric genes may be composed of sequences from two or more related viral proteins of different viral species.

The AAV vector of the invention may take the form of a nucleotide sequence comprising
10 an AAV genome or derivative thereof and a sequence encoding the Complement Factor I, and/or Complement Factor H or FHL1 transgene or derivatives thereof.

The AAV particles of the invention include transcapsidated forms wherein an AAV genome or derivative having an ITR of one serotype is packaged in the capsid of a different serotype. The AAV particles of the invention also include mosaic forms wherein
15 a mixture of unmodified capsid proteins from two or more different serotypes makes up the viral capsid. The AAV particle also includes chemically modified forms bearing ligands adsorbed to the capsid surface. For example, such ligands may include antibodies for targeting a particular cell surface receptor.

Thus, for example, the AAV particles of the invention include those with an AAV2 genome
20 and AAV2 capsid proteins (AAV2/2), those with an AAV2 genome and AAV5 capsid proteins (AAV2/5) and those with an AAV2 genome and AAV8 capsid proteins (AAV2/8), as well as those with an AAV2 genome and capsid proteins of more than one serotype.

The AAV vector may comprise multiple copies (e.g., 2, 3 etc.) of the nucleotide sequence referred to herein.

25 In some embodiments, the polynucleotide further comprises one or more AAV ITRs. In preferred embodiments, the polynucleotide further comprises two AAV ITRs. In some embodiments, the polynucleotide comprises an AAV ITR at its 5' end and an AAV ITR at its 3' end. In some embodiments, the AAV ITRs are AAV2 or AAV8 ITRs. In preferred embodiments, the AAV ITRs are AAV2 ITRs.

30 In some embodiments, the polynucleotide comprises a 5' AAV ITR with the nucleotide sequence of SEQ ID NO: 18, or a nucleotide sequence that has at least 75%, 80%, 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereto.

CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCCTCAGTGAGC
GAGCGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTTCT

5 SEQ ID NO: 18

In some embodiments, the polynucleotide further comprises the nucleotide sequence of SEQ ID NO: 20, or a nucleotide sequence that has at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereto, immediately adjacent to the 3' end of the 5' ITR.

10 TGTAGTTAATGATTAACCCGCCATGCTACTTATCTACGTAGCCATGCTCTAGGTACC

SEQ ID NO: 20

In some embodiments, the polynucleotide comprises a 3' AAV ITR with the nucleotide sequence of SEQ ID NO: 19, or a nucleotide sequence that has at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereto.

AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAA
AGGTCGCCCGACGCCGGGCTTTGCCCGGGCGGCTCAGTGAGCGAGCG

15 SEQ ID NO: 19

In some embodiments, the polynucleotide further comprises the nucleotide sequence of SEQ ID NO: 21, or a nucleotide sequence that has at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity thereto, immediately adjacent to the 5' end of the 3' ITR.

CTTCTGAGGCGGAAGAACCAGCTGGGGCTCGACTAGAGCATGGCTACGTAGATAAGTAGCATGGCGGGTTA
ATCATTAAC TACA

25 SEQ ID NO: 21

Promoters and regulatory sequences

The polynucleotide or vector of the invention may also include elements allowing for the expression of the Complement Factor I and CFI cofactor, such as Complement Factor H and/or FHL1 transgenes in vitro or in vivo. These may be referred to as expression control sequences. Thus, the polynucleotide or vector typically comprises expression control sequences (e.g. comprising a promoter sequence) operably linked to the nucleotide sequence encoding the transgene.

In some embodiments, the polynucleotide or vector comprises nucleotide sequences encoding: (a) a CMV promoter, optionally wherein the CMV promoter is upstream of the nucleotide sequences encoding the Complement Factor I and/or a Complement Factor I (CFI) cofactor, such as Complement Factor H and/or FHL1; (b) a WPRE regulatory element, optionally wherein the WPRE regulatory element is downstream of the nucleotide sequences encoding the Complement Factor I and/or a Complement Factor I (CFI) cofactor, such as Complement Factor H and/or FHL1; and/or (c) a poly-A signal, such as a Bovine Growth Hormone poly-A signal, optionally wherein the poly-A signal is downstream of the nucleotide sequences encoding the Complement Factor I and/or a Complement Factor I (CFI) cofactor, such as Complement Factor H and/or FHL1.

In preferred embodiments, the polynucleotide or vector comprises:

- (a) a 5' AAV ITR;
- (b) a CMV promoter;
- (c) a nucleotide sequence encoding a Complement Factor I (CFI) cofactor, preferably FHL1;
- (d) a linker, optionally wherein the linker comprises or is defined by a Furin cleavage site, GSG, 11a1D and an F2A sequence;
- (e) a nucleotide sequence encoding CFI;
- (f) a WPRE regulatory element, preferably wherein the WPRE regulatory element is a WPRE3 regulatory element;
- (g) a Bovine Growth Hormone poly-A signal; and
- (h) a 3' AAV ITR.

Any suitable promoter may be used, the selection of which may be readily made by the skilled person. The promoter sequence may be constitutively active (i.e. operational in any host cell background), or alternatively may be active only in a specific host cell environment, thus allowing for targeted expression of the transgene in a particular cell type (e.g. a tissue-specific promoter). The promoter may show inducible expression in response to presence of another factor, for example a factor present in a host cell. In any event, where the vector is administered for therapy, it is preferred that the promoter should be functional in the target cell background.

In some embodiments, it is preferred that the promoter shows retinal-cell specific expression in order to allow for the transgene to only be expressed in retinal cell populations. Thus, expression from the promoter may be retinal-cell specific, for example confined only to cells of the neurosensory retina and retinal pigment epithelium.

- 5 Preferred promoters, which are not retinal-cell specific, include the chicken beta-actin (CBA) promoter, optionally in combination with a cytomegalovirus (CMV) enhancer element. An example promoter for use in the invention is a CAG promoter, for example the promoter used in the rAVE expression cassette (GeneDetect.com).

In preferred embodiments, the polynucleotide or vector comprises a CMV promoter.

- 10 An example CMV promoter sequence is:

GGAGTTCCGCGTTACATAAATTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATTGAC
GTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTT
ACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGA
15 CGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGA
CTCACGGGGATTTCGAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTGTTTGGCACCAAAATCAACGGGA
CTTTCCAAAATGTCGTAACTCCGCCCATTTGACGCAAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTA
TATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTGGACCTCCATAG
AAGACACCG

20 (SEQ ID NO: 13)

In some embodiments, the polynucleotide or vector comprises a promoter with a nucleotide sequence that has at least 75%, 80%, 85% 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 13. Preferably, wherein the nucleotide sequence substantially retains the functional activity of the promoter represented by SEQ ID NO: 13.

- 25 In other embodiments, the polynucleotide or vector comprises a promoter with the nucleotide sequence of SEQ ID NO: 13.

A further example promoter sequence is:

ATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGA
GTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGT
30 CAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTA
CATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTC
CCCCCCTCCCCACCCCAATTTTGTATTTATTTATTTTAAATATTTTGTGACGCGATGGGGGCGGGGG
GGGGGGGGGGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGC
GGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGCCCTATAA
35 AAAGCGAAGCGCGCGGGGCGGGGAGTTCGCTGCGCGCTGCCCTTCGCCCCGTGCCCCGCTCCGCCGCGCCTC
GCGCCGCCCGCCCCGCTCTGACTGACCGGCTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCC
GGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTCCTTTCTGTGGCTGCGTGAAAGCCTTGAGGGCT
CCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGCTGTCCGCGGGGGACGGCTGCCCTTCGGGGGGGAC
40 GGGGACAGGCGGGGTTGCGCTTCTGGCGTGTGACCGCGGCTCTAGAGCCTCTGCTAACCATTGTTTCATGCC
TCTTCTTTTCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATATTTTGGCAAGAAT

(SEQ ID NO: 5)

In some embodiments, the polynucleotide or vector comprises a promoter with a nucleotide sequence that has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 5. Preferably, wherein the nucleotide sequence substantially retains the functional activity of the promoter represented by SEQ ID NO: 5.

In other embodiments, the polynucleotide or vector comprises a promoter with the nucleotide sequence of SEQ ID NO: 5.

Examples of promoters based on human sequences that would induce retina-specific gene expression include rhodopsin kinase for rods and cones (Allocca et al. (2007) J. Virol. 81: 11372-80), PR2.1 for cones only (Mancuso et al. (2009) Nature 461: 784-7) and/or RPE65 (Bainbridge et al. (2008) N. Engl. J. Med. 358: 2231-9) or VMD2 (Esumi et al. (2004) J. Biol. Chem. 279: 19064-73) for the retinal pigment epithelium.

The polynucleotide or vector of the invention may also comprise one or more additional regulatory sequences which may act pre- or post-transcriptionally. The regulatory sequence may be part of the native transgene locus or may be a heterologous regulatory sequence. The polynucleotide or vector of the invention may comprise portions of the 5'-UTR or 3'-UTR from the native transgene transcript.

Regulatory sequences are any sequences which facilitate expression of the transgene, i.e. act to increase expression of a transcript, improve nuclear export of mRNA or enhance its stability. Such regulatory sequences include for example enhancer elements, post-transcriptional regulatory elements and polyadenylation sites.

A preferred polyadenylation site is the Bovine Growth Hormone poly-A (bGH poly-A) signal.

An example Bovine Growth Hormone poly-A (bGH poly-A) signal is:

GTGCCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCTCCCCCGTGCCCTTCCTTGACCCTGGAAGGTGCCACT
CCCACTGTCCTTTTCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGG
GGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGC
TCTATGG

(SEQ ID NO: 14)

A further example Bovine Growth Hormone poly-A (bGH poly-A) signal is:

TCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCTCCCCCGTGCCCTTCCTT
GACCCTGGAAGGTGCCACTCCCACTGTCCTTTTCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAG
GTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCA
TGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCACTGGGG

(SEQ ID NO: 6)

In some embodiments, the polynucleotide or vector comprises a polyadenylation signal with a nucleotide sequence that has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 14 or 6, preferably SEQ ID NO: 14. Preferably, wherein the nucleotide sequence substantially retains the functional activity of the polyadenylation signal represented by SEQ ID NO: 14 or 6.

In other embodiments, the polynucleotide or vector comprises a polyadenylation signal with the nucleotide sequence of SEQ ID NO: 14 or 6, preferably SEQ ID NO: 14.

In the context of the polynucleotide or vector of the invention, such regulatory sequences will be cis-acting. However, the invention also encompasses the use of trans-acting regulatory sequences located on additional genetic constructs.

A preferred post-transcriptional regulatory element for use in a AAV vector of the invention is the woodchuck hepatitis post-transcriptional regulatory element (WPRE) or a variant thereof.

An example WPRE is:

15 ATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTAT
GTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGT
ATAAATCCTGGTTGCTGCTCTTTATGAGGAGTTGTGGCCCGTTGTGTCAGGCAACGTGGCGTGGTGTGCACTG
TGTGTTGCTGACGCAACCCCACTGGTTGGGGCATGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTT
20 TCCCCCTCCCTATTGCCACGGCGGAACATCATCGCCGCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGT
TGGGCACTGACAATCCGTTGGTGTGTGTCGGGGAAATCATCGTCTTTCCTTGGCTGCTCGCCTGTGTTGCCA
CCTGGATTCTGCGCGGGACGTCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCG
GCCTGTGCGCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGG
CCGCTCCCCGC

(SEQ ID NO: 7)

25 WPRE is a tripartite element containing gamma, alpha and beta elements, in the given order. A shortened version of WPRE, which contains only minimal gamma and alpha elements (referred to as WPRE3; Choi, J.-H. et al. (2014) Molecular Brain 7: 17), may also be used in the invention.

An example WPRE3 sequence is:

30 AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTA
TGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTG
TATAAATCCTGGTTAGTTCTTGCCACGGCGGAACATCATCGCCGCTGCCTTGCCCGCTGCTGGACAGGGGCT
CGGCTGTTGGGCACTGACAATCCGTTGGT

(SEQ ID NO: 15)

In some embodiments, the polynucleotide or vector comprises a post-transcriptional regulatory element with a nucleotide sequence that has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 15 or 7, preferably SEQ ID NO: 15. Preferably, wherein the nucleotide sequence substantially retains the functional activity of the post-transcriptional regulatory element represented by SEQ ID NO: 15 or 7.

In other embodiments, the polynucleotide or vector comprises a post-transcriptional regulatory element with the nucleotide sequence of SEQ ID NO: 15 or 7, preferably SEQ ID NO: 15.

Another regulatory sequence which may be used in a polynucleotide or vector of the invention is a scaffold-attachment region (SAR). Additional regulatory sequences may be readily selected by the skilled person.

METHOD OF ADMINISTRATION

The products, polynucleotide or vector of the invention may be administered systemically (for example by peripheral vein infusion) and may be administered locally or regionally (for example to the CNS system by intrathecal injection). In preferred embodiments, the product, polynucleotide or vector is administered intraocularly.

The term "intraocular" refers to the interior of the eye, thus intraocular administration relates to the administration to the interior of the eye of a subject

In some embodiments, the product, polynucleotide or vector is administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection. In some embodiments, said administration is performed by a robot.

The volume of the medicament composition injected may, for example, be about 10-500 μL , for example about 50-500, 100-500, 200-500, 300-500, 400-500, 50-250, 100-250, 200-250 or 50-150 μL . The volume may, for example, be about 10, 50, 100, 150, 200, 250, 300, 350, 400, 450 or 500 μL . Preferably, the volume of the medicament composition injected is 100 μL .

The skilled person will be familiar with and well able to carry out individual subretinal, direct retinal, suprachoroidal or intravitreal injections.

Preferably, the product, polynucleotide or vector is administered by subretinal injection.

In some embodiments, the product, polynucleotide, vector or pharmaceutical composition comprising the same is administered not more than once, or not more than twice, during the lifetime of a subject.

Subretinal injection

- 5 Subretinal injections are injections into the subretinal space, i.e. underneath the neurosensory retina. During a subretinal injection, the injected material is directed into, and creates a space between, the photoreceptor cell and retinal pigment epithelial (RPE) layers.

When the injection is carried out through a small retinotomy, a retinal detachment may be created. The detached, raised layer of the retina that is generated by the injected material is referred to as a “bleb”.

10

The hole created by the subretinal injection must be sufficiently small that the injected solution does not significantly reflux back into the vitreous cavity after administration. Such reflux would be particularly problematic when a medicament is injected, because the effects of the medicament would be directed away from the target zone. Preferably, the injection

15 creates a self-sealing entry point in the neurosensory retina, i.e. once the injection needle is removed, the hole created by the needle reseals such that very little or substantially no injected material is released through the hole.

To facilitate this process, specialist subretinal injection needles are commercially available (e.g. DORC 41G Teflon subretinal injection needle, Dutch Ophthalmic Research Center International BV, Zuidland, The Netherlands). These are needles designed to carry out subretinal injections.

20

Unless damage to the retina occurs during the injection, and as long as a sufficiently small needle is used, substantially all injected material remains localised between the detached neurosensory retina and the RPE at the site of the localised retinal detachment (i.e. does not reflux into the vitreous cavity). Indeed, the typical persistence of the bleb over a short time frame indicates that there is usually little escape of the injected material into the vitreous. The bleb may dissipate over a longer time frame as the injected material is absorbed.

25

Visualisations of the eye, in particular the retina, for example using optical coherence tomography, may be made pre-operatively.

- 30 The volume of the medicament composition injected may, for example, be about 10-500 μL , for example about 50-500, 100-500, 200-500, 300-500, 400-500, 50-250, 100-250, 200-250 or 50-150 μL . The volume may, for example, be about 10, 50, 100, 150, 200, 250, 300, 350,

400, 450 or 500 μL . Preferably, the volume of the medicament composition injected is 100 μL . Larger volumes may increase the risk of stretching the retina, while smaller volumes may be difficult to see.

Two-step subretinal injection

- 5 The product, polynucleotide or vector of the invention may be delivered with increased accuracy and safety by using a two-step method in which a localised retinal detachment is created by the subretinal injection of a first solution. The first solution does not comprise the product, polynucleotide or vector. A second subretinal injection is then used to deliver the medicament comprising the product, polynucleotide or vector into the subretinal fluid of the
- 10 bleb created by the first subretinal injection. Because the injection delivering the medicament is not being used to detach the retina, a specific volume of solution may be injected in this second step.

In some embodiments, the subretinal injection of the vector comprises the steps:

- 15 (a) administering a solution to the subject by subretinal injection in an amount effective to at least partially detach the retina to form a subretinal bleb, wherein the solution does not comprise the product, polynucleotide or vector; and
- (b) administering a medicament composition by subretinal injection into the bleb formed by step (a), wherein the medicament comprises the product,
- 20 polynucleotide or vector.

The volume of solution injected in step (a) to at least partially detach the retina may be, for example, about 10-1000 μL , for example about 50-1000, 100-1000, 250-1000, 500-1000, 10-500, 50-500, 100-500, 250-500 μL . The volume may be, for example, about 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 μL .

- 25 The volume of the medicament composition injected in step (b) may be, for example, about 10-500 μL , for example about 50-500, 100-500, 200-500, 300-500, 400-500, 50-250, 100-250, 200-250 or 50-150 μL . The volume may be, for example, about 10, 50, 100, 150, 200, 250, 300, 350, 400, 450 or 500 μL . Preferably, the volume of the medicament composition injected in step (b) is 100 μL . Larger volumes may increase the risk of stretching the retina,
- 30 while smaller volumes may be difficult to see.

The solution that does not comprise the medicament (i.e. the "solution" of step (a)) may be similarly formulated to the solution that does comprise the medicament, as described below.

A preferred solution that does not comprise the medicament is balanced saline solution (BSS) or a similar buffer solution matched to the pH and osmolality of the subretinal space.

Visualising the retina during surgery

Under certain circumstances, for example during end-stage retinal degenerations, identifying the retina is difficult because it is thin, transparent and difficult to see against the disrupted and heavily pigmented epithelium on which it sits. The use of a blue vital dye (e.g. Brilliant Peel®, Geuder; MembraneBlue-Dual®, Dorc) may facilitate the identification of the retinal hole made for the retinal detachment procedure (i.e. step (a) in the two-step subretinal injection method of the invention) so that the medicament can be administered through the same hole without the risk of reflux back into the vitreous cavity.

The use of the blue vital dye also identifies any regions of the retina where there is a thickened internal limiting membrane or epiretinal membrane, as injection through either of these structures would hinder clean access into the subretinal space. Furthermore, contraction of either of these structures in the immediate post-operative period could lead to stretching of the retinal entry hole, which could lead to reflux of the medicament into the vitreous cavity.

Suprachoroidal injection

The product, polynucleotide or vector of the invention may be delivered to the suprachoroidal space using an ab externo approach that utilises a microcatheter (see, for example, Peden et al. (2011) PLoS One 6(2): e17140). In this method a limbal conjunctival peritomy is performed to expose bare sclera, followed by sclerotomy to expose bare choroid. A microcatheter (such as the iTrack 250A from iScience Interventional, optionally connected to an illumination system such as the iLumin laser-diode based micro-illumination system (iScience Interventional)) is introduced into the suprachoroidal space and advanced posteriorly towards the optic disc. Following manipulation of the microcatheter tip into the desired position, injection of the product, polynucleotide or vector forms a bleb within the retina and choroid.

Thus, in some embodiments, the product, polynucleotide or vector is delivered suprachoroidally by a method comprising (i) introduction of a microcatheter into the suprachoroidal space; (ii) advancing the microcatheter within said space until the tip is in the proximity of the afflicted region of the retina; and (iii) injecting the product, polynucleotide or vector from the microcatheter tip to create a bleb.

In some embodiments, the above administration procedures are directly carried out by a robot.

PHARMACEUTICAL COMPOSITIONS AND INJECTED SOLUTIONS

The medicaments, for example products, polynucleotides or vectors, of the invention may be formulated into pharmaceutical compositions. These compositions may comprise, in addition to the medicament, a pharmaceutically acceptable carrier, diluent, excipient, buffer, stabiliser or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may be determined by the skilled person according to the route of administration, e.g. subretinal, direct retinal, suprachoroidal or intravitreal injection.

The pharmaceutical composition is typically in liquid form. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, magnesium chloride, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. In some cases, a surfactant, such as pluronic acid (PF68) 0.001% may be used.

For injection at the site of affliction, the active ingredient may be in the form of an aqueous solution which is pyrogen-free, and has suitable pH, isotonicity and stability. The skilled person is well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection or Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included as required.

For delayed release, the medicament may be included in a pharmaceutical composition which is formulated for slow release, such as in microcapsules formed from biocompatible polymers or in liposomal carrier systems according to methods known in the art.

METHOD OF TREATMENT

It is to be appreciated that all references herein to treatment include curative, palliative and prophylactic treatment; although in the context of the invention references to preventing are more commonly associated with prophylactic treatment. Treatment may also include arresting progression in the severity of a disease.

The treatment of mammals, particularly humans, is preferred. However, both human and veterinary treatments are within the scope of the invention.

The term "combination", or terms "in combination", "used in combination with" or "combined preparation" as used herein may refer to the combined administration of two or more agents simultaneously, sequentially or separately.

5 The term "simultaneous" as used herein means that the agents are administered concurrently, i.e. at the same time.

The term "sequential" as used herein means that the agents are administered one after the other.

10 The term "separate" as used herein means that the agents are administered independently of each other but within a time interval that allows the agents to show a combined, preferably synergistic, effect. Thus, administration "separately" may permit one agent to be administered, for example, within 1 minute, 5 minutes or 10 minutes after the other.

VARIANTS, DERIVATIVES, ANALOGUES, HOMOLOGUES AND FRAGMENTS

15 In addition to the specific proteins and nucleotides mentioned herein, the invention also encompasses the use of variants, derivatives, analogues, homologues and fragments thereof.

20 In the context of the invention, a variant of any given sequence is a sequence in which the specific sequence of residues (whether amino acid or nucleic acid residues) has been modified in such a manner that the polypeptide or polynucleotide in question substantially retains its function. A variant sequence can be obtained by addition, deletion, substitution, modification, replacement and/or variation of at least one residue present in the naturally-occurring protein.

25 The term "derivative" as used herein, in relation to proteins or polypeptides of the invention includes any substitution of, variation of, modification of, replacement of, deletion of and/or addition of one (or more) amino acid residues from or to the sequence providing that the resultant protein or polypeptide substantially retains at least one of its endogenous functions.

The term "analogue" as used herein, in relation to polypeptides or polynucleotides includes any mimetic, that is, a chemical compound that possesses at least one of the endogenous functions of the polypeptides or polynucleotides which it mimics.

30 Typically, amino acid substitutions may be made, for example from 1, 2 or 3 to 10 or 20 substitutions provided that the modified sequence substantially retains the required activity or ability. Amino acid substitutions may include the use of non-naturally occurring analogues.

Proteins used in the invention may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent protein. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues as long as the endogenous function is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include asparagine, glutamine, serine, threonine and tyrosine.

Conservative substitutions may be made, for example according to the table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R H
AROMATIC		F W Y

The term "homologue" as used herein means an entity having a certain homology with the wild type amino acid sequence and the wild type nucleotide sequence. The term "homology" can be equated with "identity".

A homologous sequence may include an amino acid sequence which may be at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% identical, preferably at least 95% or 97% or 99% identical to the subject sequence. Typically, the homologues will comprise the same active sites etc. as the subject amino acid sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the invention it is preferred to express homology in terms of sequence identity.

A homologous sequence may include a nucleotide sequence which may be at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% identical, preferably at least 95% or 97% or 99% identical to the subject sequence. Although homology can also be considered in terms of similarity, in the context of the invention it is preferred to express homology in terms of sequence identity.

Preferably, reference to a sequence which has a percent identity to any one of the SEQ ID NOs detailed herein refers to a sequence which has the stated percent identity over the entire length of the SEQ ID NO referred to.

5 Homology comparisons can be conducted by eye or, more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate percentage homology or identity between two or more sequences.

Percentage homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is
10 called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion in the nucleotide sequence may cause the following codons to be put out of alignment, thus
15 potentially resulting in a large reduction in percent homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

20 However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible, reflecting higher relatedness between the two compared sequences, will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller
25 penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package the default gap penalty for amino
30 acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum percentage homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux et al. (1984) *Nucleic Acids Res.* 12: 387). Examples of other

software that can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al. (1999) *ibid* – Ch. 18), FASTA (Atschul et al. (1990) *J. Mol. Biol.* 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel et al. (1999) *ibid*, pages 7-58 to 7-60).

- 5 However, for some applications, it is preferred to use the GCG Bestfit program. Another tool, called BLAST 2 Sequences is also available for comparing protein and nucleotide sequences (see *FEMS Microbiol. Lett.* (1999) 174: 247-50; *FEMS Microbiol. Lett.* (1999) 177: 187-8).

- 10 Although the final percent homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix – the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a
15 custom symbol comparison table if supplied (see the user manual for further details). For some applications, it is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

- Once the software has produced an optimal alignment, it is possible to calculate percent homology, preferably percent sequence identity. The software typically does this as
20 part of the sequence comparison and generates a numerical result.

- “Fragments” of full length Complement Factor I or Complement Factor I (CFI) cofactor, such as Complement Factor H or FHL1 are also variants and the term typically refers to a selected region of the polypeptide or polynucleotide that is of interest either functionally or, for example, in an assay. “Fragment” thus refers to an amino acid or nucleic acid sequence
25 that is a portion of a full-length polypeptide or polynucleotide.

- Such variants may be prepared using standard recombinant DNA techniques such as site-directed mutagenesis. Where insertions are to be made, synthetic DNA encoding the insertion together with 5' and 3' flanking regions corresponding to the naturally-occurring sequence either side of the insertion site may be made. The flanking regions will contain
30 convenient restriction sites corresponding to sites in the naturally-occurring sequence so that the sequence may be cut with the appropriate enzyme(s) and the synthetic DNA ligated into the cut. The DNA is then expressed in accordance with the invention to make the encoded protein. These methods are only illustrative of the numerous standard techniques known in the art for manipulation of DNA sequences and other known techniques may also be used.

The skilled person will understand that they can combine all features of the invention disclosed herein without departing from the scope of the invention as disclosed.

Preferred features and embodiments of the invention will now be described by way of non-limiting examples.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, biochemistry, molecular biology, microbiology and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press; Ausubel, F.M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, Ch. 9, 13 and 16, John Wiley & Sons; Roe, B., Crabtree, J. and Kahn, A. (1996) DNA Isolation and Sequencing: Essential Techniques, John Wiley & Sons; Polak, J.M. and McGee, J.O'D. (1990) In Situ Hybridization: Principles and Practice, Oxford University Press; Gait, M.J. (1984) Oligonucleotide Synthesis: A Practical Approach, IRL Press; and Lilley, D.M. and Dahlberg, J.E. (1992) Methods in Enzymology: DNA Structures Part A: Synthesis and Physical Analysis of DNA, Academic Press. Each of these general texts is herein incorporated by reference.

EXAMPLES

EXAMPLE 1

Cofactor assay

Recombinant Complement Factor I (CFI), cofactor (Complement Factor H (CFH) or Complement Factor H-like Protein 1 (FHL1)) and C3b were incubated together for 20 min at 37°C.

Concentrations of CFI and C3b were fixed, and titrations of CFH or FHL1 in specified ratios were carried out.

The cleavage of C3b to iC3b was quantified by ELISA.

The results of these studies are shown in Figure 2A.

From these results, it may be concluded that a minimum functional CFI:CFH/FHL1 molar ratio is 1:2.

Measurement of normal serum CFI:cofactor ratios

Complement Factor I (CFI), Complement Factor H (CFH) and Complement Factor H-like Protein 1 (FHL1) concentrations were measured in normal human serum using ELISAs.

The molar ratio of CFI:cofactor in normal serum was found to be 1:8.3.

5 Comparison of plasma and ocular fluid CFI:cofactor ratios

We subsequently compared the levels of CFI and CFH in both blood plasma and ocular fluids. Data showed that in contrast to plasma, in which the cofactor exists in several fold molar excess to CFI enzyme, this ratio is reversed in vitreous and aqueous humor (Figure 7).

10 Complement deposition assay on lipopolysaccharide (LPS)

A microtitre plate was coated with 1 µg/well of LPS and incubated overnight at 4°C.

The plate was then washed and incubated for 1 h at 37°C with normal human serum supplemented with differing amounts of recombinant Complement Factor I (CFI), Complement Factor H (CFH) or Complement Factor H-like Protein 1 (FHL1). The plate was subsequently washed thoroughly, and complement deposition was measured with a mouse anti-C3d antibody (Abcam) and donkey anti-mouse (Jackson ImmunoResearch) used as a secondary antibody.

Figure 2B shows the effect of CFI, CFH and FHL1 supplementation on complement deposition on LPS.

20 The x-axes show the ratio of Complement Factor I (CFI) to Complement Factor H + Complement Factor H like 1 (CFH and FHL1, the “cofactors”). Changes in the ratio were achieved solely by addition of recombinant CFI, CFH or FHL1.

25 Although the starting molar ratio of 1:8.3 (the natural ratio in normal serum) already has excess cofactor compared to enzyme, a further benefit on reduction of complement deposition and therefore activation is achieved when the ratio is further raised. This additional benefit may be related to an additional function (decay-accelerating activity, DAA) of the CFH and FHL1. Both CFH and FHL1 have DAA in that they compete with Complement Factor B (FB) for C3b binding, dislocate FB from C3b and thereby “decay” the alternative pathway convertase.

From these experiments, it is possible to conclude that below a minimum CFI:CFH/FHL1 molar ratio of 1:2, the cofactor becomes limiting.

EXAMPLE 2

Generation of bicistronic plasmids

- 5 Recombinant AAV transgene plasmids (termed RC204, RC206-210 and RC212-218) were constructed comprising AAV2 5' and 3' inverted terminal repeats (ITRs) flanking the cassettes described in Table 1.

Table 1

RC number	5' ITR Adjacent Sequence	Promoter	Transgene #1	Linker	Transgene #2	WPRES	PolyA	3' ITR Adjacent Sequence	Size (bp)
RC204	Yes	CMV	CFI	IRES-CC	FHL-1	No	BGHpA	Yes	4882
RC206			CFI-CO	IRES-CC	FHL-1-CO	No			4882
RC207			CFI	IRES-PV	FHL-1	No			5069
RC208			CFI-CO	IRES-PV	FHL-1-CO	No			5069
RC209			FHL-1	Furin-F2A	CFI	Standard			5052
RC210			FHL-1-CO	Furin-F2A	CFI-CO	Standard			5052
RC212			FHL-1-CO	Furin-F2A	CFI-CO	3			4674
RC213	No		CFI	IRES-CC	FHL-1	No		No	4744
RC214			CFI	IRES-PV	FHL-1	No			4929
RC215			FHL-1	Furin-F2A	CFI	Standard			4912
RC216			FHL-1-CO	Furin-F2A	CFI-CO	Standard			4912
RC217			FHL-1	Furin-F2A	CFI	3			4548
RC218			FHL-1-CO	Furin-F2A	CFI-CO	3			4548

The 5' ITR adjacent sequence used was SEQ ID NO: 20.

- 10 The CMV promoter sequence used was SEQ ID NO: 13.

The FHL1 sequence used was SEQ ID NO: 16. The codon optimised FHL1 (FHL1-CO) sequence used was SEQ ID NO: 12.

The Furin-F2A linker sequence used was SEQ ID NO: 17.

- 15 The CFI sequence used was SEQ ID NO: 2. The codon optimised CFI (CFI-CO) sequence used was SEQ ID NO: 10.

The WPRES3 sequence used was SEQ ID NO: 15.

The Bovine growth hormone poly-A (BGHpA) sequence used was SEQ ID NO: 14.

The 3' ITR adjacent sequence used was SEQ ID NO: 21.

The overall sequences of RC212 and RC218 are SEQ ID NOs: 22 and 23, respectively.

Comparison of vectors by transduction

5 Vector production in adherent HEK293

Separate transfection HEK293 cells with 12 plasmids (RC204, RC206-210 and RC212-217) was carried out using the following protocol:

Day 1: HEK293 cells were dissociated and counted using a ViCell. Cells were seeded in 10 cm plates at 6×10^5 cells per cm^2 in 10 mL DMEM/Glutamax + 10% FBS per plate.

10 Day 2: Confluency was checked and was found to be between 70-80%.

Media was replaced with DMEM/Glutamax + 5% FBS

After 4 hours, cells were transfected with 5 μg total plasmid DNA (1.25 μg construct DNA, 1.25 μg RepCap plasmid, 2.5 μg Helper plasmid) per plate using PEI at a 1:3 DNA:PEI ratio in duplicates:

- 15
1. 2x 5 μg DNA was diluted in 2x 1 mL PBS.
 2. 2x 15 μL PEI was added and incubated for 20 min.
 3. 1 mL of DNA/PEI was added dropwise to the cells.

Day 3: 15 mM of sodium butyrate was added.

20 Day 5: Media were harvested by pooling the duplicates, and centrifuged at 1000 rpm for 10 minutes to remove cell debris.

The supernatant was transferred to a new tube and AAVanced (AAV110A-1, Cambridge Bioscience) was added at 1/5 of the volume.

The mixture was incubated at 4°C for 72h.

25 Day 5: The mixture was centrifuged at 1000 rpm for 30 minutes at 4°C. Subsequently the supernatant was discarded, and the pellet was resuspended in 500 μL of PBS. The pellet was centrifuged for 3 minutes at 1500 g and then the supernatant was discarded again. The

pellet was resuspended in 1/100 of the original supernatant volume and stored at -80°C until titration.

Transduction of HEK293 cells using vectors

Separate transduction of HEK293 cells with 12 viral vectors (RC204, RC206-210 and
5 RC212-217) was carried out using the following protocol:

Day 1: HEK293 cells were dissociated and counted using a ViCell. Cells were seeded in 24 well plates at 1×10^5 cells in 400 μ L DMEM/Glutamax + 10% FBS per well.

Day 2: The required amount of viral vector was added to achieve a multiplicity of infection (MOI) of 2×10^2 .

10 Day 3: Media were removed and replaced with 300 μ L of serum free DMEM/Glutamax.

Day 5: The supernatant was harvested and centrifuged at 14000 rpm at 4°C. The clarified supernatant was transferred in new tubes ready for analysis.

Western blot

Supernatants from the transduction were analysed by Western blot (primary antibodies to
15 CFI and FHL-1; and ECL Prime Western blotting detection reagents were used).

The Western blot analyses are shown in Figure 3.

CFI ELISA

Supernatants from the transduction were analysed by ELISA for CFI using the following procedure:

20 Day 1: An ELISA plate was coated with 50 μ L per well sheep anti-CFI polyclonal antibody diluted 1 in 4000 in 1x coating buffer. Plates were stored at 4°C overnight.

Day 2: The plate was washed 3 times with 200 μ L per well PBS-Tween (0.05%) then blotted on a tissue.

200 μ L 1% BSA fraction V in PBS-Tween (0.05%) was applied to each well and allowed to
25 block for 2 hours at room temperature.

Samples and standard curves were prepared during the blocking incubation. A standard curve was prepared using purified CFI protein (Sigma C5938-1MG) diluted in DMEM 2% FBS. Samples were diluted 1:5 and 1:10 in DMEM 2% FBS.

After 2 hours blocking, the plate was washed 3 times, as described above, then 50 µL sample or standard was loaded onto each well and incubated at room temperature for 1 hour.

5 After 1 hour, the plate was washed as above, then anti-CFI (Ox21) antibody was diluted 1 in 2000 in DMEM 5% FBS and 50 µL of this was applied to each well and incubated at room temperature for 1 hour.

After 1 hour, the plate was washed as above then donkey anti-mouse-HRP antibody was diluted 1 in 5000 in DMEM 5% FBS and 50 µL of this was applied to each well and incubated at room temperature for 1 hour.

10 After 1 hour, the plate was washed as above, then 100 µL TMB reagent was applied to each well and incubated at room temperature in the dark for approximately 15 minutes. Once sufficient blue colour had been obtained, 100 µL 1 M sulphuric acid was added to each well to stop the reaction.

15 The A450 was then recorded with and data were processed and transferred to Microsoft Excel for analysis.

FHL1 ELISA

Supernatants from the transfection were analysed by ELISA for FHL1 using the following procedure:

20 Day 1: An ELISA plate was coated with 50 µL per well, 3 µg/mL anti-FHL-1 antibody (Biorad, AbD33594.1) in 100 mM Carbonate/Bicarbonate Buffer, pH 9.6. Plates stored at 4°C overnight.

Day 2: The plate was washed 3 times with 200 µL per well TBS-Tween (0.05%) then blotted on a tissue.

25 200 µL 1% BSA fraction V in PBS-Tween (0.05%) was applied to each well and allowed to block for 2 hours at room temperature.

Samples and standard curves were prepared during the blocking incubation. A standard curve was prepared using FHL1 purified protein diluted in DMEM 2% FBS. Samples were diluted 1:5 and 1:10 in blocking buffer.

After 2 hours blocking, the plate was washed 3 times, as described above, then 50 µL sample or standard was loaded onto each well and incubated at room temperature for 1 hour.

- 5 After 1 hour, the plate was washed as above, then anti-CFH antibody (Ox24, Santa Cruz Biotechnologies, sc-53067) was added at 0.33 µg/mL in blocking buffer and 50 µL of this was applied to each well and incubated at room temperature for 1 hour.

After 1 hour, the plate was washed as above then donkey anti-mouse-HRP antibody was added at 0.2 µg/mL in blocking buffer and 50 µL of this was applied to each well and incubated at room temperature for 1 hour.

- 10 After 1 hour, the plate was washed as above, then 100 µL TMB reagent was applied to each well and incubated at room temperature in the dark for approximately 15 minutes. Once sufficient blue colour had been obtained, 50 µL 1 M sulphuric acid was added to each well to stop the reaction.

- 15 The A450 was then recorded with and data were processed and transferred to Microsoft Excel for analysis.

Conclusions

The results of the Western blot and ELISA studies are shown in Figures 3 and 4, respectively.

From the Western blot and ELISA analyses, it may be concluded that:

- 20 All candidates produce both CFI and FHL1 after transduction.

CFI and FHL1 codon optimisation increases protein levels.

RC206 and RC212 are optimal for CFI expression, however RC212 achieves optimal CFI:FHL1 molar ratio (>1:2).

RC212 is the best candidate based on CFI/FHL1 expression.

- 25 **Vector packaging**

Alkaline gel analysis

24 µL of each undiluted sample and SRM control (2.45×10^{11} vg/mL; SRM #16-048) were loaded onto a 0.8% alkaline gel, which was then run for 19 hours at 20 V in a cold room in alkaline running buffer (40 ml 50x alkaline buffer + 1960 mL MilliQ water).

5 The gel was then incubated in 3 gel vols of 0.1 M Tris pH 8.0 for 1 h at room temperature, then in 1 gel volume 0.1 M NaCl containing 4x SYBRGold nucleic acid stain for 2 h at room temperature (protected from light exposure), then rinsed twice in MilliQ water.

The gel was then visualised using SYBRGold UV transilluminator setting on a Chemidoc at 10 seconds exposure time.

The result is shown in Figure 5 (top panel).

10 *Full:empty particle ratio*

The ratio of full to empty viral particles was analysed by comparing titres as calculated from qPCR (DNA titre) and capsid ELISA.

The result is shown in Figure 5 (bottom panel).

Conclusions

15 From these analyses it may be concluded that:

Efficient packaging only with RC212.

There appears to be incomplete packaging of genomes >4.7kb.

Full-empty particle ratio of RC212 is comparable to the control monocistronic vectors.

RC212 is the best candidate based on packaging analysis.

20 **C3b cleavage assay**

In the C3b cleavage assay, 1 mg of plasma purified C3b is incubated for 1 hour at 37°C with transduced HEK293 supernatant samples.

Analyses were carried out using ELISA, or Western blot analysis (described as follows). 4x Laemmli buffer with β-mercaptoethanol was added to stop the reaction. Samples were
25 diluted and loaded on a 10% precast polyacrylamide SDS PAGE gel (Bio-Rad). After transfer to a PVDF membrane (Bio-Rad) and blocking in blocking buffer (1xTBS pH 8 [Sigma]/0.05% Tween-20 and 5% dried skimmed milk powder [Marvel]), C3b cleavage was detected using goat anti-human C3 antibody (Biorad).

The results are shown in Figure 6, from which it is possible to conclude that RC212 is the most potent.

EXAMPLE 3 – ADDITIONAL COMPLEMENT DOWN-REGULATION ON ADDITION OF MULTIPLE COMPLEMENT REGULATORS

5 Methods

To measure functional activity of complement regulators, an LPS deposition assay was performed. Nunc Maxisorb plates were coated overnight at 4°C with 1 µg/mL LPS (Sigma, Escherichia coli O26:B6) in diluted ELISA Coating Buffer (BioRad, BUF030B). Plates were washed with PBS-0.05% Tween 20. 25% Serum in alternative pathway buffer (PBS, 2 mM
10 MgCl₂ and 10 mM EGTA, pH 7.2) was prepared and supplemented with complement regulators. Dilutions were added to the LPS-coated plate and incubated for 1 hour at 37°C. 10 mM EDTA was added to a separate tube with serum to prevent complement activation and this sample was used to determine background signal in the assay. The plate was washed as before and complement activation measured by detecting C3 deposition on the
15 plate (goat anti-C3d Abcam, ab17453; 1:20,000). After 1 hour incubation at ambient temperature, plates were washed and incubated for another hour with donkey anti-mouse HRP conjugated antibody (Jackson ImmunoResearch, 715-035-150; 1:1,000). After four washes, the plates were incubated with 1-Step Ultra TMB - ELISA Substrate (Life Technologies) and the reaction was quenched with 1 M H₂SO₄. The OD at 450 nm was
20 measured using a Varioskan plate reader (Thermo Fisher) and the IC₅₀ was determined from 4PL fitted curves using GraphPad Prism. Complement preserved female human serum was used for these experiments.

Results

The IC₅₀ concentration of complement regulator proteins, Complement Factor I, soluble
25 complement receptor 1, Complement Factor H or Factor H-like 1, was determined in a separate experiment using the same assay platform. Here, results show (Figure 8) that when the IC₅₀ concentrations of regulator proteins is added, the alternative pathway (as measured by reduction of C3 deposition) is quenched (shaded columns). If Complement Factor I is co-supplemented with one of its cofactors (soluble complement receptor 1, complement Factor
30 H or Factor H-like 1), additional quenching is observed (white columns), demonstrating that increasing the concentration of two complement regulators is superior to addition of either alone. These results indicate that conditions caused by an overreactive complement system might benefit from a dual administration of multiple complement regulators.

The IC₅₀ concentrations of the individual regulators differ widely across the regulators tested (sCR1 ~10x more potent than FHL1 and ~50x more potent than CFH and CFI) but also in their molecular weight (sCR1=213kDa, CFI=88kDa, CFH=155kDa and FHL1=49kDa), molar concentrations were used for this direct comparison. Endogenous levels of proteins will also contribute to observed differences in potency; Factor H has a much higher plasma concentration than FHL1 or sCR1. By comparing the ability of regulators at their IC₅₀ concentration to quench the alternative pathway, it was demonstrated that sCR1 is the most potent regulator because it requires the lowest molar concentration to achieve the IC₅₀. Because CR1 is a membrane bound receptor on erythrocytes, which are abundant in blood but not serum/plasma, and only minute amounts of fluid CR1 (i.e. sCR1) are present in serum or plasma, this infers that sCR1 would be a very potent complement regulator and CFI cofactor at sites where erythrocytes are separated from plasma, such as the choroidal space, Bruch's membrane subretinal space and the glomerulus.

15 **EXAMPLE 4 – EXPRESSION OF COMPLEMENT FACTOR I AND FACTOR H-LIKE 1 IN VITRO EXPRESSION**

Methods

HEK-293 cells were transduced with one of the following rAAV vectors: AAV expressing CFI (GT005); AAV expressing FHL1 (RC001); or AAV expressing CFI and FHL1 (GT007). Supernatants were analysed by non-reducing western blot to determine relative protein expression. Goat anti human CFI (Comptech) and goat anti human FH (Quidel, A312) were used as primary antibodies to detect CFI and FHL1 protein.

Results

Western blot analysis showed that CFI and FHL1 protein were expressed and secreted into the culture medium from all three constructs (Figure 9A and B). To confirm this pattern of expression, supernatants were analysed by immunoblotting to visualise CFI protein to determine correct processing and secretion in the supernatant. As demonstrated in Figure 9B, heavy and light chain CFI, as well as pro-CFI, are secreted from HEK-293 cells following transduction with GT007, confirming that CFI protein was translated, and that proteolytic processing has occurred. In mammalian cells transfected with plasmid encoding CFI cDNA, not all of the recombinant pro-CFI protein undergoes cleavage, resulting in secretion of pro-CFI (88 kDa) as well as the mature processed CFI, consisting of the 50 kDa heavy chain and the 38 kDa light chain.

EXAMPLE 5 – FUNCTIONALITY OF COMPLEMENT FACTOR I AND FACTOR H-LIKE 1 IN VITRO EXPRESSION

Methods

To analyse functional activity of CFI and FHL1 secreted from transduced cells, conditioned supernatant from HEK-293 cells transduced with: AAV expressing CFI (GT005) alone; AAV2 expressing FHL1 (RC001) alone; co-transduced with both GT005 and RC001; or transduced with AAV expressing CFI and FHL1 (GT007) was tested in a C3b cleavage assay (Figure 10). In this assay, C3b is mixed together with a source of CFI and FHL1 and incubated for 4 hours at 37°C. This incubation time was optimised for the concentrations of transgenes expressed from the transduced cells. The principle of this assay is based on the ability of CFI, in the presence of FHL1, to cleave C3b into iC3b and C3f. The assay is analysed by a C3b western blot, staining the C3b cleavage products, and by iC3b ELISA which quantifies the amount of the C3b breakdown product, iC3b.

Results

Results of both the C3 western blot and iC3b ELISA correlate and demonstrate functional activity of CFI and FHL1 expressed in vitro. In the C3b western blot (Figure 10A), lane 1 shows C3b only, lane 2 shows C3b mixed with CFI and FHL1 (positive control) and lane 3 shows C3b mixed with conditioned supernatant from untransduced cells (UTD, negative control). Lane 4 shows C3b degradation when conditioned supernatant of cells co-transduced with GT005 (CFI) and RC001 (FHL1). Lane 5 shows conditioned supernatant of cells transduced with GT007 (expressing CFI and FHL1). The assay confirms that conditioned supernatant of GT007-transduced cells contains active CFI and FHL1 that degrade C3b into iC3b.

The iC3b ELISA (Figure 10B) was performed using the same supernatant used for the C3b western blot and the amount of iC3b was quantified as a direct function of protein functionality. As before, C3b incubated with CFI and FHL1 acts as a positive control as does conditioned supernatant of GT005 and RC001 co-transduced cells. The supernatant of GT007-transduced cells shows C3b cleavage activity, confirming presence of active CFI and FHL1.

EXAMPLE 6 – IN VIVO EFFICACY IN A MOUSE MODEL OF CHOROIDAL NEOVASCULARISATION (CNV)

Methods

A laser-induced choroidal neovascularisation model was performed in mice. Mice (n = 12-14 per group) received unilateral subretinal injections of AAV vectors 4 weeks prior to the CNV induction, or aflibercept (positive control) just after the CNV induction. The contralateral eye served as control. Mice were followed using in vivo imaging, fluorescein angiography (FA) and spectral-domain optical coherence tomography (SD-OCT), at days 4 and 7. At the end of the study period at the follow-up day 7 after the CNV induction, the mice were sacrificed by anaesthesia overdose, serum was collected and the eyes were enucleated. Neural retina was excised and fresh frozen in liquid nitrogen. Choroids were post-fixed and choroidal flatmounts were prepared. Histological analysis from choroidal flat mounts was used to quantify the area of isolectin B4 staining in the CNV lesions.

Results

The aflibercept group significantly affected the degree of CNV leakage compared to the Null vector control group at 4 days post-CNV induction ($P < 0.0001$ compared to all other groups), but this was lost by Day 7, presumably due to drug washout over time. The area of CNV leakage was significantly decreased in aflibercept-treated eyes both at Day 4 ($P < 0.0001$ for all group comparisons) and Day 7 ($P = 0.019$ vs. null vector group).

Choroidal flat-mounts were co-stained with fluorescein-labelled isolectin. Isolectin B4 stains endothelial cells and is used to visualise CNV lesions. Data were non-normally distributed as assessed by Kolmogorov-Smirnov test ($P < 0.05$) and therefore, statistical significance of the observed differences was determined using Generalized Linear Model (GLM) analysis. All treatment groups showed statistically significant reduction in the isolectin B4-stained area as compared to the null treatment group (GLM, $P < 0.05$ for all). Co-administration of CFI and FHL1-expressing vectors (GT005:RC001) provided the most significant reduction.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the disclosed agents, compositions, uses and methods of the invention will be apparent to the skilled person without departing from the scope and spirit of the invention. Although the invention has been disclosed in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the disclosed modes for carrying out the invention, which are obvious to the skilled person are intended to be within the scope of the following claims.

The invention is further described by the following numbered paragraphs:

1. A product comprising (i) Complement Factor H Like Protein 1 (FHL1) or Complement Factor H (CFH); and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor, as a combined preparation for simultaneous, separate or sequential use in therapy.
5
2. A product comprising (i) Complement Factor H Like Protein 1 (FHL1) or Complement Factor H (CFH); and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor, as a combined preparation for simultaneous, separate or sequential use in treating or preventing a complement-mediated disorder of the eye.
- 10 3. The product for use according to para 2, wherein the complement-mediated disorder is age-related macular degeneration (AMD) or diabetic retinopathy, preferably AMD.
4. The product for use according to para 3, wherein the AMD is dry AMD.
5. The product for use according to any preceding para, wherein the product provides (i) and (ii) to a subject in a (i):(ii) molar ratio of at least 2:1, preferably at least 3:1,
15 more preferably at least 8:1, more preferably at least 15:1.
6. The product for use according to any preceding para, wherein the product provides (i) and (ii) to a subject in a (i):(ii) molar ratio of between 2:1 and 12:1, preferably between 3:1 and 10:1.
7. An isolated polynucleotide comprising nucleotide sequences encoding (i)
20 Complement Factor H Like Protein 1 (FHL1) or Complement Factor H (CFH); and (ii) Complement Factor I (CFI).
8. The isolated polynucleotide of para 7, wherein the polynucleotide further comprises nucleotide sequences encoding:
 - 25 (a) a CMV promoter, optionally wherein the CMV promoter is upstream of the nucleotide sequences encoding the (i) and (ii);
 - (b) a WPRE regulatory element, optionally wherein the WPRE regulatory element is downstream of the nucleotide sequences encoding the (i) and (ii); and/or

- (c) a poly-A signal, optionally a Bovine Growth Hormone poly-A signal, wherein the polyA signal is optionally downstream of the nucleotide sequences encoding the (i) and (ii).
9. The isolated polynucleotide of para 7 or 8, wherein the nucleotide sequence encoding (i) is upstream of the nucleotide sequence encoding (ii).
10. The isolated polynucleotide of any one of paras 7-9, wherein the polynucleotide further comprises one or more adeno-associated virus (AAV) inverted terminal repeats (ITRs).
11. The isolated polynucleotide of any one of paras 7-10, wherein the polynucleotide comprises an AAV ITR at its 5' end and an AAV ITR at its 3' end.
12. The isolated polynucleotide of any one of paras 7-11, wherein the polynucleotide comprises:
- (a) a 5' AAV ITR;
 - (b) a CMV promoter;
 - (c) a nucleotide sequence encoding FHL1 or CFH, preferably FHL1;
 - (d) a linker, optionally wherein the linker is comprises or is defined by a Furin cleavage site, GSG, 11aa1D sequence and an F2A sequence;
 - (e) a nucleotide sequence encoding CFI;
 - (f) a WPRE regulatory element, optionally wherein the WPRE regulatory element is a WPRE3 regulatory element;
 - (g) a Bovine Growth Hormone poly-A signal; and
 - (h) a 3' AAV ITR.
13. The isolated polynucleotide of any one of paras 7-12, wherein the AAV ITRs are AAV2 or AAV8 ITRs.
14. The isolated polynucleotide of any one of paras 7-13, wherein the nucleotide sequences encoding FHL1 or CFH, and CFI are codon optimised.
15. The isolated polynucleotide of any one of paras 7-14, wherein: (a) the nucleotide sequence encoding FHL1 has at least 75% sequence identity to SEQ ID NO: 12;

and/or (b) the nucleotide sequence encoding CFI has at least 75% sequence identity to SEQ ID NO: 10.

- 5
16. The isolated polynucleotide of any one of paras 7-15, wherein: (a) the nucleotide sequence encoding FHL1 is SEQ ID NO: 12; and/or (b) the nucleotide sequence encoding CFI is SEQ ID NO: 10.
17. The isolated polynucleotide of any one of paras 7-16, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 22 or 23, or a nucleotide sequence that has at least 75% sequence identity thereto.
- 10 18. The isolated polynucleotide of any one of paras 7-17, wherein the polynucleotide is less than or equal to 4.7 kb.
19. A vector comprising the polynucleotide of any one of paras 7-18.
20. The vector of para 19, wherein the vector is an adeno-associated viral (AAV) vector.
21. The vector of para 19 or 20, wherein the vector is in the form of a viral vector particle.
- 15 22. The vector of para 21, wherein the AAV vector particle comprises an AAV2 or AAV8 genome, and AAV2 or AAV8 capsid proteins.
23. A cell comprising the polynucleotide of any one of paras 7-18.
24. A cell transduced with the vector of any one of paras 19-22.
- 20 25. A pharmaceutical composition comprising the polynucleotide, vector or cell of any one of paras 7-24 in combination with a pharmaceutically acceptable carrier, diluent or excipient.
26. The polynucleotide, vector or cell of any one of paras 7-24 for use in therapy.
27. The polynucleotide, vector or cell of any one of paras 7-24 for use in treating or preventing a complement-mediated disorder of the eye.
- 25 28. The polynucleotide, vector or cell for use according to para 27, wherein the complement-mediated disorder is age-related macular degeneration (AMD) or diabetic retinopathy, preferably AMD.
29. The polynucleotide, vector or cell for use according to para 28, wherein the AMD is dry AMD.

30. The polynucleotide, vector or cell for use according to any one of paras 26-29, wherein the formation of geographic atrophy is prevented or reduced, and/or the amount of geographic atrophy is reduced.
31. The polynucleotide, vector or cell for use according to any one of paras 26-30,
5 wherein the progression of geographic atrophy is slowed.
32. The polynucleotide, vector or cell for use according to any one of paras 26-31, wherein there is at least a 10% reduction in the increase in geographic atrophy area over the 12 months following administration to a treated eye of a subject, relative to an untreated eye over the same period.
- 10 33. The polynucleotide, vector or cell for use according to any one of paras 26-32, wherein administration of the polynucleotide, vector or cell increases the level of C3b-inactivating and iC3b-degradation activity in a subject, or in an eye, such as in the retinal pigment epithelium (RPE), of a subject, optionally to a level that exceeds a normal level in a subject, or eye or RPE thereof.
- 15 34. The polynucleotide, vector or cell for use according to any one of paras 26-33, wherein the polynucleotide, vector or cell is administered intraocularly.
35. The polynucleotide, vector or cell for use according to any one of paras 26-34, wherein the polynucleotide, vector or cell is administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection.
- 20 36. The polynucleotide, vector or cell for use according to any one of paras 26-35, wherein the polynucleotide, vector or cell is administered to the eye of a subject by subretinal injection.
37. A method of treating or preventing a complement-mediated disorder of the eye comprising administering the polynucleotide, vector or cell of any one of paras 7-24
25 to a subject in need thereof.
38. A method of providing (i) Complement Factor H Like Protein 1 (FHL1) or Complement Factor H (CFH); and (ii) Complement Factor I (CFI) to a subject, comprising delivering the polynucleotide, vector or cell of any one of paras 7-24 to the eye of the subject.

CLAIMS

1. A product comprising (i) a Complement Factor I (CFI) cofactor; and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor, as a combined preparation for simultaneous, separate or sequential use in therapy.
- 5 2. A product comprising (i) a Complement Factor I (CFI) cofactor; and (ii) Complement Factor I (CFI), or nucleotide sequences encoding therefor, as a combined preparation for simultaneous, separate or sequential use in treating or preventing a complement-mediated disorder, preferably a complement-mediated disorder of the eye.
- 10 3. The product for use according to claim 2, wherein the complement-mediated disorder is age-related macular degeneration (AMD) or diabetic retinopathy, preferably AMD.
4. The product for use according to claim 3, wherein the AMD is dry AMD.
5. The product for use according to any preceding claim, wherein the Complement Factor I (CFI) cofactor is selected from the group consisting of Complement Factor H Like Protein 1 (FHL1); Complement Factor H (CFH); Complement Receptor 1 (CR1)
- 15 or a fragment thereof; and Membrane Cofactor Protein (MCP) or a fragment thereof.
6. The product for use according to any preceding claim, wherein the product provides (i) and (ii) to a subject in a (i):(ii) molar ratio of at least 2:1, preferably at least 3:1, more preferably at least 8:1, more preferably at least 15:1.
- 20 7. The product for use according to any preceding claim, wherein the product provides (i) and (ii) to a subject in a (i):(ii) molar ratio of between 2:1 and 12:1, preferably between 3:1 and 10:1.
8. An isolated polynucleotide comprising nucleotide sequences encoding (i) a Complement Factor I (CFI) cofactor; and (ii) Complement Factor I (CFI).
- 25 9. The isolated polynucleotide of claim 8, wherein the polynucleotide further comprises nucleotide sequences encoding:
 - (a) a CMV promoter, optionally wherein the CMV promoter is upstream of the nucleotide sequences encoding the (i) and (ii);

- (b) a WPRE regulatory element, optionally wherein the WPRE regulatory element is downstream of the nucleotide sequences encoding the (i) and (ii); and/or
- (c) a poly-A signal, optionally a Bovine Growth Hormone poly-A signal, wherein the polyA signal is optionally downstream of the nucleotide sequences encoding the (i) and (ii).
10. The isolated polynucleotide of claim 8 or 9, wherein the nucleotide sequence encoding (i) is upstream of the nucleotide sequence encoding (ii).
11. The isolated polynucleotide of any one of claims 8-10, wherein the polynucleotide further comprises one or more adeno-associated virus (AAV) inverted terminal repeats (ITRs).
12. The isolated polynucleotide of any one of claims 8-11, wherein the polynucleotide comprises an AAV ITR at its 5' end and an AAV ITR at its 3' end.
13. The isolated polynucleotide of any one of claims 8-12, wherein the polynucleotide comprises:
- (a) a 5' AAV ITR;
- (b) a CMV promoter;
- (c) a nucleotide sequence encoding a Complement Factor I (CFI) cofactor;
- (d) a linker, optionally wherein the linker is or is defined by a Furin cleavage site, GSG, 11aa1D sequence and an F2A sequence;
- (e) a nucleotide sequence encoding CFI;
- (f) a WPRE regulatory element, optionally wherein the WPRE regulatory element is a WPRE3 regulatory element;
- (g) a Bovine Growth Hormone poly-A signal; and
- (h) a 3' AAV ITR.
14. The isolated polynucleotide of any one of claims 8-13, wherein the Complement Factor I (CFI) cofactor is selected from the group consisting of Complement Factor H

Like Protein 1 (FHL1); Complement Factor H (CFH); Complement Receptor 1 (CR1) or a fragment thereof; and Membrane Cofactor Protein (MCP) or a fragment thereof.

15. The isolated polynucleotide of any one of claims 8-14, wherein the AAV ITRs are AAV2 or AAV8 ITRs.
- 5 16. The isolated polynucleotide of any one of claims 8-15, wherein the nucleotide sequences encoding the CFI cofactor, and CFI are codon optimised.
17. The isolated polynucleotide of any one of claims 8-16, wherein: (a) the nucleotide sequence encoding FHL1 has at least 75% sequence identity to SEQ ID NO: 12; and/or (b) the nucleotide sequence encoding CFI has at least 75% sequence identity to SEQ ID NO: 10.
- 10 18. The isolated polynucleotide of any one of claims 8-17, wherein: (a) the nucleotide sequence encoding FHL1 is SEQ ID NO: 12; and/or (b) the nucleotide sequence encoding CFI is SEQ ID NO: 10.
19. The isolated polynucleotide of any one of claims 8-18, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 22 or 23, or a nucleotide sequence that has at least 75% sequence identity thereto.
- 15 20. The isolated polynucleotide of any one of claims 8-19, wherein the polynucleotide is less than or equal to 4.7 kb.
21. A vector comprising the polynucleotide of any one of claims 8-20.
- 20 22. The vector of claim 21, wherein the vector is an adeno-associated viral (AAV) vector.
23. The vector of claim 21 or 22, wherein the vector is in the form of a viral vector particle.
24. The vector of claim 23, wherein the AAV vector particle comprises an AAV2 or AAV8 genome, and AAV2 or AAV8 capsid proteins.
- 25 25. A cell comprising the polynucleotide of any one of claims 8-20.
26. A cell transduced with the vector of any one of claims 21-24.
27. A pharmaceutical composition comprising the polynucleotide, vector or cell of any one of claims 8-26 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

28. The polynucleotide, vector or cell of any one of claims 8-26 for use in therapy.
29. The polynucleotide, vector or cell of any one of claims 8-26 for use in treating or preventing a complement-mediated disorder, preferably a complement-mediated disorder of the eye.
- 5 30. The polynucleotide, vector or cell for use according to claim 29, wherein the complement-mediated disorder is age-related macular degeneration (AMD) or diabetic retinopathy, preferably AMD.
31. The polynucleotide, vector or cell for use according to claim 30, wherein the AMD is dry AMD.
- 10 32. The polynucleotide, vector or cell for use according to any one of claims 28-31, wherein the formation of geographic atrophy is prevented or reduced, and/or the amount of geographic atrophy is reduced.
33. The polynucleotide, vector or cell for use according to any one of claims 28-32, wherein the progression of geographic atrophy is slowed.
- 15 34. The polynucleotide, vector or cell for use according to any one of claims 28-33, wherein there is at least a 10% reduction in the increase in geographic atrophy area over the 12 months following administration to a treated eye of a subject, relative to an untreated eye over the same period.
- 20 35. The polynucleotide, vector or cell for use according to any one of claims 28-34, wherein administration of the polynucleotide, vector or cell increases the level of C3b-inactivating and iC3b-degradation activity in a subject, or in an eye, such as in the retinal pigment epithelium (RPE), of a subject, optionally to a level that exceeds a normal level in a subject, or eye or RPE thereof.
- 25 36. The polynucleotide, vector or cell for use according to any one of claims 28-35, wherein the polynucleotide, vector or cell is administered intraocularly.
37. The polynucleotide, vector or cell for use according to any one of claims 28-36, wherein the polynucleotide, vector or cell is administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection.
- 30 38. The polynucleotide, vector or cell for use according to any one of claims 28-37, wherein the polynucleotide, vector or cell is administered to the eye of a subject by subretinal injection.

39. A method of treating or preventing a complement-mediated disorder, preferably a complement-mediated disorder of the eye comprising administering the polynucleotide, vector or cell of any one of claims 8-26 to a subject in need thereof.
40. A method of providing (i) a Complement Factor I (CFI) cofactor; and (ii) Complement Factor I (CFI) to a subject, comprising delivering the polynucleotide, vector or cell of any one of claims 8-26 to the subject, preferably to the eye of the subject.
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