

NUCLEIC ACID ENCODING AN ANTI-VEGF ENTITY AND A NEGATIVE COMPLEMENT REGULATOR AND USES THEREOF FOR THE TREATMENT OF AGE-RELATED MACULAR DEGENERATION

FIELD OF THE INVENTION

The present invention relates to agents for use in gene therapy. In particular, the invention relates to combinations of an anti-VEGF entity and a negative complement regulator, or
5 nucleotide sequences 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).

BACKGROUND TO THE INVENTION

The macula is a small area in the retina of the eye, approximately 3 to 5 millimetres in size,
10 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.

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). The wet form of AMD is associated with
15 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).
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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.
25 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
30 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 has been classified into “dry” and “wet” (exudative or neovascular) forms. 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, which represent the late advanced stages of dry AMD. Patients with this form of AMD 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 into the subretinal space in 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. Polypoidal choroidal vasculopathy (PCV) is also known to occur.

Current treatment options for wet AMD are principally a number of therapies which target the Vascular Endothelial Growth Factor (VEGF) pathway. Examples of such VEGF-targeted therapies include the aptamer pegaptanib (N Engl J Med (2004) 351: 2805-2816) and antibodies such as ranibizumab (N Engl J Med (2006) 355: 1432-1444), aflibercept (BMJ (2014) 98: i17-i21) and bevacizumab (BMJ (2010) 340: c2459). However, not all patients respond to treatment with an anti-VEGF antibody and either do not recover vision or progress to registered blindness. An incremental decline of visual acuity (VA) gains achieved with monthly treatment has also been reported (Singer *et al.*; Ophthalmology; 2012; 119(6); 1175-83).

Further, there is evidence to support an association between anti-VEGF therapy and progression of geographic atrophy (Gemenetza *et al.*; Eye; 2017; 31; 1-9, Enslow *et al.*; Ophthalmology and Eye Diseases; 2016; 8; 21-32 & Sadda *et al.*; Ophthalmology; 2020; 127(5); 648-659).

There is also a need for a therapeutic strategy to reduce drug burden to patients and address problems with adherence and/or underdosing.

Accordingly, there is a significant need in the art for new approaches to treat eye diseases, such as AMD.

SUMMARY OF THE INVENTION

Without wishing to be bound by theory, the present invention is based – at least in part – on the insight that it may be advantageous to target underlying mechanisms typically associated with “dry” AMD at the same time as targeting mechanisms typically associated with “wet” AMD.

- 5 In addition, the invention is based in part on consideration of how the therapeutic targeting of one form of AMD may impact the other.

In a first aspect, the present invention provides a product comprising (i) an anti-VEGF entity; and (ii) a negative complement regulator, or nucleotide sequences encoding therefor, as a combined preparation for simultaneous, separate or sequential use in therapy.

- 10 In one embodiment, the product may be one or more polynucleotides which between them encode (i) an anti-VEGF entity; and (ii) a negative complement regulator.

Thus in a preferred embodiment, there is provided a polynucleotide that encodes an anti-VEGF entity and a negative complement regulator.

- 15 Preferably, the polynucleotide is a bicistronic polynucleotide encoding an anti-VEGF entity and a negative complement regulator.

- In particular, the applicant has provided vectors that can be used for the delivery of both an anti-VEGF entity and a negative complement regulator 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 an anti-VEGF entity and a negative complement regulator.
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- The anti-VEGF entity may be selected from an Ig fusion protein, an antibody, an aptamer, a polypeptide, a peptide, a polynucleotide (e.g. an antisense oligonucleotide, siRNA, shRNA, CRISPRi guidestrand of a sequence that targets the VEGF gene sequence or that of its mRNA) and a non-antibody scaffold. Suitably, the anti-VEGF entity is selected from
- 25 aflibercept, ranibizumab, bevacizumab, brolucizumab and pegaptanib or a fragment or variant thereof.

- The negative complement regulator may be selected from Complement Factor I (CFI), Complement Factor H Like Protein 1 (FHL1), Complement Factor H (CFH), Complement receptor type 1 (CR1), Membrane Cofactor Protein (MCP), Complement decay-accelerating factor (DAF) and MAC-inhibitory protein (MAC-IP), C1-inhibitor, anaphylatoxins inhibitor, C4b binding protein (C4BP), clusterin, vitronectin or a fragment or variant thereof.
- 30

In some embodiments, the polynucleotide further comprises a nucleotide sequence encoding a CMV promoter or a chicken β -actin promoter. Preferably, the CMV or CAG promoter is upstream of the nucleotide sequences encoding the anti-VEGF entity and the negative complement regulator. The chicken β -actin promoter may be provided in combination with a
5 CMV early enhancer (i.e. as a CAG promoter).

In some embodiments, the polynucleotide further comprises a nucleotide sequence encoding a WPRE regulatory element. Preferably, the WPRE regulatory element is downstream of the nucleotide sequences encoding the anti-VEGF entity and the negative complement regulator.

In some embodiments, the polynucleotide further comprises a nucleotide sequence encoding
10 a poly-A signal. Preferably, wherein the poly-A signal is downstream of the nucleotide sequences encoding the anti-VEGF entity and the negative complement regulator.

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 anti-VEGF entity and
15 the negative complement regulator.

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

- (a) a CMV promoter or CAG promoter, wherein the CMV promoter or CAG promoter is upstream of the nucleotide sequences encoding the anti-VEGF entity and the negative complement regulator; and
- 20 (b) a Bovine Growth Hormone poly-A signal, wherein the Bovine Growth Hormone poly-A signal is downstream of the nucleotide sequences encoding the anti-VEGF entity and the negative complement regulator.

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

- 25 (a) a CMV promoter or CAG promoter, wherein the CMV promoter or CAG promoter is upstream of the nucleotide sequences encoding the anti-VEGF entity and the negative complement regulator;
- (b) a WPRE regulatory element, wherein the WPRE regulatory element is downstream of the nucleotide sequences encoding the anti-VEGF entity and the negative complement regulator; and

- (c) a Bovine Growth Hormone poly-A signal, wherein the Bovine Growth Hormone poly-A signal is downstream of the nucleotide sequences encoding the anti-VEGF entity and the negative complement regulator.

The WPRE regulatory element may be a WPRE3 regulatory element.

- 5 The nucleotide sequence encoding the anti-VEGF entity may be upstream of the nucleotide sequence encoding the negative complement regulator.

In some embodiments, the nucleotide sequence encoding the anti-VEGF entity is upstream of the nucleotide sequence encoding the negative complement regulator, wherein the anti-VEGF entity is selected from aflibercept, ranibizumab, bevacizumab, brolucizumab and pegaptanib
10 or a fragment or variant thereof. In some embodiments, the nucleotide sequence encoding the anti-VEGF entity is upstream of the nucleotide sequence encoding the negative complement regulator, wherein the anti-VEGF entity is aflibercept or a fragment or variant thereof. In some embodiments, the nucleotide sequence encoding the anti-VEGF entity is upstream of the nucleotide sequence encoding the negative complement regulator, wherein the negative
15 complement regulator is CFI or a variant or fragment thereof. In some embodiments, the nucleotide sequence encoding the anti-VEGF entity is upstream of the nucleotide sequence encoding the negative complement regulator, wherein the negative complement regulator is FHL1 or a variant or fragment thereof.

In some embodiments, the nucleotide sequence encoding the anti-VEGF entity is upstream of
20 the nucleotide sequence encoding the negative complement regulator, wherein the anti-VEGF entity is aflibercept or a fragment or variant thereof and the negative complement regulator is CFI or a variant or fragment thereof.

In some embodiments, the nucleotide sequence encoding the anti-VEGF entity is upstream of the nucleotide sequence encoding the negative complement regulator, wherein the anti-VEGF
25 entity is aflibercept or a fragment or variant thereof and the negative complement regulator is FHL1 or a variant or fragment thereof.

The nucleotide sequence encoding the negative complement regulator may be upstream of the nucleotide sequence encoding the anti-VEGF entity.

In some embodiments, the nucleotide sequence encoding the negative complement regulator
30 is upstream of the nucleotide sequence encoding the anti-VEGF entity, wherein the anti-VEGF entity is selected from aflibercept, ranibizumab, bevacizumab, brolucizumab and pegaptanib or a fragment or variant thereof. In some embodiments, the nucleotide sequence encoding the

negative complement regulator is upstream of the nucleotide sequence encoding the anti-VEGF entity, wherein the anti-VEGF entity is aflibercept or a fragment or variant thereof. In some embodiments, the nucleotide sequence encoding the negative complement regulator is upstream of the nucleotide sequence encoding the anti-VEGF entity, wherein the negative complement regulator is CFI or a variant or fragment thereof. In some embodiments, the nucleotide sequence encoding the negative complement regulator is upstream of the nucleotide sequence encoding the anti-VEGF entity, wherein the negative complement regulator is FHL1 or a variant or fragment thereof.

In some embodiments, the nucleotide sequence encoding the negative complement regulator is upstream of the nucleotide sequence encoding the anti-VEGF entity, wherein the anti-VEGF entity is aflibercept or a fragment or variant thereof and the negative complement regulator is CFI or a variant or fragment thereof.

In some embodiments, the nucleotide sequence encoding the negative complement regulator is upstream of the nucleotide sequence encoding the anti-VEGF entity, wherein the anti-VEGF entity is aflibercept or a fragment or variant thereof and the negative complement regulator is FHL1 or a variant or fragment thereof.

In some embodiments, the nucleotide sequences encoding the anti-VEGF entity and the negative complement regulator are operably linked by a linker. In some embodiments, the linker is a Furin, GSG, 11aa1D and 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.

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.

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 or a CAG promoter;
- (c) a nucleotide sequence encoding an anti-VEGF entity;
- (d) a linker, optionally wherein the linker comprises a Furin cleavage site, GSG, 11a1D and an F2A sequence;
- (e) a nucleotide sequence encoding a negative complement regulator;

- (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;
- 5 (b) a CMV promoter or a CAG promoter;
- (c) a nucleotide sequence encoding an anti-VEGF entity;
- (d) a linker, optionally wherein the linker comprises a Furin cleavage site, GSG, 11a1D and an F2A sequence;
- (e) a nucleotide sequence encoding a negative complement regulator;
- 10 (f) a WPRE regulatory element, optionally 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.

Suitably the polynucleotides defined by (c) and (e) may be in the reciprocal position in the
15 polynucleotide.

In particular, in some embodiments, the polynucleotide comprises:

- (a) a 5' AAV ITR;
- (b) a CMV promoter or a CAG promoter;
- (c) a nucleotide sequence encoding a negative complement regulator;
- 20 (d) a linker, optionally wherein the linker comprises a Furin cleavage site, GSG, 11a1D and an F2A sequence;
- (e) a nucleotide sequence encoding an anti-VEGF entity;
- (f) a poly-A signal, preferably a Bovine Growth Hormone poly-A signal; and
- (g) a 3' AAV ITR.

25 In some embodiments, the polynucleotide comprises:

- (a) a 5' AAV ITR;
- (b) a CMV promoter or a CAG promoter;
- (c) a nucleotide sequence encoding a negative complement regulator;
- (d) a linker, optionally wherein the linker comprises a Furin cleavage site, GSG,
30 11a1D and an F2A sequence;
- (e) a nucleotide sequence encoding an anti-VEGF entity;
- (f) a WPRE regulatory element, optionally 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.

Suitably, in embodiments where (b) comprises a CAG promoter; (f) may be a WPRE3 regulatory element.

The polynucleotide may comprise (a)-(g) or (a)-(h) in order, from 5' to 3'.

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

In some embodiments, the nucleotide sequences encoding the anti-VEGF entity and/or negative complement regulator are codon optimised. In some embodiments, the nucleotide sequence encoding the anti-VEGF entity is codon optimised. In some embodiments, the
10 nucleotide sequence encoding the negative complement regulator is codon optimised. In preferred embodiments, the nucleotide sequences encoding aflibercept and CFI, FHL1 or CFH are codon optimised.

In some embodiments, the nucleotide sequence encoding aflibercept has at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity
15 to SEQ ID NO: 11.

In preferred embodiments, the nucleotide sequence encoding aflibercept is SEQ ID NO: 11.

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: 35 or 36.

- 20 In preferred embodiments, the nucleotide sequence encoding CFI is SEQ ID NO: 35 or 36.

In some embodiments, the nucleotide sequence encoding aflibercept is SEQ ID NO: 11 and the nucleotide sequence encoding CFI is SEQ ID NO: 35 or 36.

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
25 SEQ ID NO: 41.

In preferred embodiments, the nucleotide sequence encoding FHL1 is SEQ ID NO: 41.

In some embodiments, the nucleotide sequence encoding aflibercept is SEQ ID NO: 11 and the nucleotide sequence encoding FHL1 is SEQ ID NO: 41.

In some embodiments, the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 45, 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: 46, 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 comprises the nucleotide sequence of SEQ ID NO: 47, 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.

10 In some embodiments, the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 48, 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 comprises the nucleotide sequence of SEQ ID NO: 49, 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.

15 In some embodiments, the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 50, 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 comprises the nucleotide sequence of SEQ ID NO: 51, 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.

20 In some embodiments, the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 52, 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.

25 In some embodiments, the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 53, 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 comprises the nucleotide sequence of SEQ ID NO: 54, 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.

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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.

In another aspect, the invention provides a vector comprising the polynucleotide of the invention. Thus according to one aspect the invention provides a vector comprising (i) polynucleotide encoding an anti-VEGF entity and (ii) a polynucleotide encoding a negative complement regulator. Preferably the anti-VEGF entity and the negative complement regulator are provided on the same polynucleotide, which is preferably a bicistronic polynucleotide.

Suitably, a bicistronic polynucleotide as used herein may refer to a polynucleotide comprising at least two protein coding regions each operably linked to the same promoter region. Suitably, the bicistronic polypeptide may comprise two protein coding regions each operably linked to the same promoter region.

Suitably, the present invention also provides more than one (e.g. two) vector which between them comprise (i) polynucleotide encoding an anti-VEGF entity and (ii) a polynucleotide encoding a negative complement regulator.

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.

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

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

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

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.

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 product, polynucleotide, vector, cell or pharmaceutical composition of the invention for use in therapy.

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

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

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

In another aspect, the invention provides a method of providing an anti-VEGF entity and a negative complement regulator to a subject, comprising delivering the product, polynucleotide,
15 vector or cell of the invention to the eye of the subject.

In particular embodiments, the product, polynucleotide, vector, cell or pharmaceutical composition of the invention 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.

20 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 5).

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, polypoidal choroidal vasculopathy, diabetic
25 macular edema, branch retinal vein occlusion or uveitis.

In preferred embodiments, the disorder is AMD.

In some embodiments, the disorder is wet AMD and/or dry AMD. In some embodiments, the product, polynucleotide, vector, cell or pharmaceutical composition is used to treat and/or prevent wet AMD and dry AMD simultaneously.

In some embodiments, the AMD is wet AMD and the use according to the invention further prevents and/or treats onset of dry AMD in said subject. In some embodiments, the AMD is dry AMD and the use according to the invention further prevents and/or treats onset of wet AMD in said subject.

- 5 In some embodiments, the 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 µg/mL in serum; and/or
- (b) being heterozygous or homozygous for an age-related macular degeneration (AMD)-associated SNP, preferably a rare Complement Factor I and/or Complement Factor H variant.

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

- 15 (a) having a normal level of Complement Factor I activity or concentration in the eye and/or serum, preferably at least 30 µg/mL, such as 30-40 µg/mL in serum; and/or
- (b) not carrying a rare Complement Factor I variant allele.

20 In another aspect, the invention provides the product, polynucleotide, vector, cell or pharmaceutical composition 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.

- 25 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
- 30 over the same period

In some embodiments, administration of the product, polynucleotide, vector, cell or pharmaceutical composition 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) aqueous humor and/or vitreous humor, 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 product, polynucleotide, vector, cell or pharmaceutical composition 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 product, 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 product, polynucleotide, vector, cell or pharmaceutical composition 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 product, 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 product, polynucleotide, vector, cell or pharmaceutical composition 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 a further aspect, the invention provides the product, polynucleotide, vector, cell or pharmaceutical composition of the invention for use in reducing or preventing neovascularization, vascular leakage or retina edema with an eye disorder, such as an eye disorder disclosed herein.

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

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

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

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

DESCRIPTION OF THE DRAWINGS

Figure 1 – Schematics depicting the design of illustrative monocistronic aflibercept vectors and AAV genome size (ITR-ITR)

10 **Figure 2** – Comparison of aflibercept expression between different codon optimised AAV2-vectors. Data show two independent experiments

Figure 3 – Representative images of Western blots performed under (A) non-reducing and (B) reducing conditions illustrating aflibercept protein expression in cultured supernatant 72 hours post-transduction. (C) Densitometric analysis of aflibercept expression under non-
15 reducing and reducing conditions from two independent experiments

Figure 4 – Schematic diagrams illustrating the design of illustrative bicistronic vectors expressing negative complement regulator and aflibercept

Figure 5 – C3b feedback (amplification) and breakdown (down-regulation) cycles of the alternative pathway of vertebrate complement (“I” = Complement Factor I; “H” = Complement
20 Factor H; “B” = Complement Factor B; and “D” = Complement Factor D).

Figure 6 - Administration of monocistronic vectors expressing CFI (GT005) or FHL1 (RC001) in a mouse model of CNV

Figure 7 - Comparison of bicistronic vector expression in HEK293 cells. Representative expression levels of (A) AAV8 bicistronic expression of Aflibercept and CFI; (B) AAV2
25 bicistronic expression of Aflibercept (left hand bar) and CFI (right hand bar) at an MOI of 5e3; (C) AAV8 bicistronic expression of Aflibercept and FHL-1 and (D) AAV2 bicistronic expression of Aflibercept (left hand bar) and FHL-1 (right hand bar) at an MOI of 5e3; (E) AAV8 bicistronic expression of Aflibercept and CFI; (F) AAV8 bicistronic expression of Aflibercept and FHL-1.

Figure 8 - Western blot analysis showing protein expression from bicistronic vectors under
30 non-reducing conditions.

Figure 9 - Binding affinity of Aflibercept. Affinities of Aflibercept expressed by monocistronic and bicistronic vectors for VEGFA, as determined by using a binding assay that measures unbound VEGFA after incubation of a fixed concentration of human VEGFA with varying concentrations of vector derived Aflibercept. IC50 value of Eylea® derived from Kd curve =
5 2.3 nM

Figure 10 - Inhibitory properties of Aflibercept. Inhibition of VEGFA-induced proliferation of HUVEC cells using varying concentration of vector derived Aflibercept in the presence of a fixed concentration of VEGFA.

Figure 11 - C3b cleavage assays for CFI and FHL1 demonstrated that CFI and FHL1
10 expressed from bicistronic vectors is functional and can facilitate breakdown of C3b to iC3b.

Figure 12 - Aflibercept mRNA expression - monocistronic aflibercept (mouse). AAV8 (monocistronic) vectors expressing anti-VEGF (Aflibercept) were administered subretinally into the right eye of nine-week-old male mice. Transgene-derived mRNA was quantified by reverse transcription and qRT-PCR.

Figure 13 - Aflibercept protein expression - monocistronic aflibercept (mouse). AAV8
15 (monocistronic) vectors expressing anti-VEGF (Aflibercept) were administered subretinally into the right eye of nine-week-old male mice. Unbound aflibercept in ocular fluids was measured using a quantitative sandwich type ELISA.

Figure 14 - Aflibercept mRNA expression - bicistronic candidates (mouse). Bicistronic AAV
20 vectors co-expressing antiVEGF (Aflibercept) and complement regulators (hCFI or FHL-1) were administered subretinally into the right eye (OD) of 10-week-old male mice. Transgene-derived mRNA was quantified by reverse transcription and qRT-PCR.

Figure 15 - Aflibercept, CFI and FHL-1 protein expression (bicistronic vectors). Bicistronic AAV vectors co-expressing antiVEGF (Aflibercept) and complement regulators (hCFI or FHL-
25 1) were administered subretinally into the right eye (OD) of 10-week-old male mice. Unbound aflibercept in ocular fluids was measured using a quantitative sandwich type ELISA. Human CFI and FHL-1 in ocular fluids was quantified using MSD's electrochemiluminescence detection technology.

Figure 16 - In vivo efficacy in murine laser-induced choroidal neovascularisation model. Five
30 groups of mice received unilateral (right eye) subretinal injections of different AAV vectors. Four weeks after administration of the vectors, CNV was induced by laser photocoagulation.

Mice were imaged at days four and seven post lasering, and then sacrificed. Quantitation of (A) CNV leakage area and (B) CNV lesion size was carried out.

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”.

VEGF

Vascular endothelial growth factor (VEGF) is a potent angiogenic protein produced by cells that stimulates the formation of blood vessels. VEGF is a sub-family of growth factors, the platelet-derived growth factor family of cystine-knot growth factors. They are involved in both vasculogenesis (the de novo formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature).

In mammals, the VEGF family comprises five members: VEGF-A, placenta growth factor (PGF), VEGF-B, VEGF-C and VEGF-D.

Suitably, the VEGF referred to herein may be VEGF-A.

There are multiple isoforms of VEGF-A that result from alternative splicing of mRNA from a single, 8-exon *VEGFA* gene. These are classified into two groups which are referred to according to their terminal exon (exon 8) splice site: the proximal splice site (denoted VEGF_{xxx}) or distal splice site (VEGF_{xxx}b). In addition, alternate splicing of exon 6 and 7 alters their heparin-binding affinity and amino acid number (in humans: VEGF₁₂₁, VEGF₁₂₁b, VEGF₁₄₅, VEGF₁₆₅, VEGF₁₆₅b, VEGF₁₈₉, VEGF₂₀₆; the rodent orthologs of these proteins contain one fewer amino acids). These domains have important functional consequences for the VEGF splice variants, as the terminal (exon 8) splice site determines whether the proteins are pro-angiogenic (proximal splice site, expressed during angiogenesis, VEGF_{xxx}) or anti-angiogenic (distal splice site, expressed in normal tissues, VEGF_{xxx}b). In addition, inclusion or exclusion of exons 6 and 7 mediate interactions with heparin sulfate proteoglycans (HSPGs) and neuropilin co-receptors on the cell surface, enhancing their ability to bind and activate the VEGF receptors (VEGFRs).

VEGF interacts with a family of 3 receptor tyrosine kinases: VEGFR1 (VEGF receptor 1), VEGFR2, and VEGFR3. VEGFA and VEGFB bind to VEGFR1, VEGFA binds to VEGFR2, and VEGFC and VEGFD bind to both VEGFR2 and VEGFR3. Placental growth factor (PGF)

primarily interacts with VEGFR1. The VEGFRs are found on a wide variety of cell types. VEGFR1, also called Flt-1 (fms-like tyrosine kinase 1), is found on vascular endothelial cells, hematopoietic stem cells, monocytes, and macrophages. VEGFR2, also called KDR (kinase insert domain) or Flk-1 (fetal liver kinase 1), is expressed on vascular and lymphatic
5 endothelial cells; VEGFR3 (also called Flt-4) is restricted to lymphatic endothelial cells. On ligand binding, VEGFRs transduce intracellular signals through a variety of mediators. In the case of VEGFR2, which is the best characterized, these include phosphatidylinositol-3 kinase (PI3K)/Akt, mitogen-activated kinases, the nonreceptor tyrosine kinase Src, as well as PLC γ (phospholipase C gamma)/PKC (protein kinase C), which promote angiogenesis,
10 lymphangiogenesis, vascular permeability, and vascular homeostasis.

VEGF-A is important in the aetiology of diabetic retinopathy (DR). The microcirculatory problems in the retina of people with diabetes can cause retinal ischaemia, which results in the release of VEGF-A, and a switch in the balance of pro-angiogenic VEGF isoforms over the normally expressed anti-angiogenic VEGF isoforms. Pro-angiogenic VEGF may then
15 cause the creation of new blood vessels in the retina and elsewhere in the eye, heralding changes that may impact vision.

VEGF-A is also important in the disease pathology of the wet form age-related macular degeneration (AMD), which is the leading cause of blindness for the elderly of the industrialized world. The vascular pathology of AMD shares certain similarities with diabetic
20 retinopathy, although the cause of disease and the typical source of neovascularization differs between the two diseases.

As used herein, an anti-VEGF entity may refer to any entity which is capable of reducing the expression and/or activity of VEGF.

As will be apparent, the anti-VEGF entity will be capable of reducing the expression and/or
25 activity of one or more pro-angiogenic isoforms of VEGF, in particular VEGF-A.

Suitably, the VEGF may be VEGFA, VEGFB and/or PGF.

Suitably, the VEGF may be VEGFA, VEGFB and PGF.

The anti-VEGF entity may be selected from one or more of the following: an antibody, an Ig fusion protein, a polypeptide, a peptide, a polynucleotide (e.g. an antisense oligonucleotide,
30 siRNA, shRNA), a small molecule, a non-antibody scaffold, an aptamer, or combinations thereof.

Antibody as used herein is intended to encompass both full antibodies and antibody fragments thereof. Suitably antibodies include, but are not limited to, monoclonal and polyclonal antibodies, engineered antibodies including chimeric, CDR-grafted and humanised antibodies, single-chain antibodies, antibody fragments and artificially selected antibodies produced using phage display or alternative techniques.

Suitable antibody fragments capable of binding to a selected target, include Fv, ScFv, F(ab') and F(ab')₂. In addition, alternatives to classical antibodies may also be used in the invention, for example "avibodies", "avimers", "anticalins", "nanobodies" and "DARPin's".

Reference to "scFv" or "single-chain variable fragment" as used herein includes molecules wherein the variable heavy (VH) and variable light chain (VL) of an antibody are linked via a flexible oligopeptide. A scFv is thus a fusion between at least one variable heavy and at least one variable light chain.

As will be apparent, in embodiments where the anti-VEGF entity and negative complement regulator are provided as an isolated polynucleotide, the anti-VEGF entity will be an entity that can be encoded by a polynucleotide. For example, the anti-VEGF entity may be an Ig fusion protein, an antibody or antigen binding fragment thereof, a polypeptide, a peptide, a polynucleotide (e.g. an antisense oligonucleotide, siRNA, shRNA, CRISPRi guidestrand of a sequence that targets the VEGF gene sequence or that of its mRNA), a non-antibody scaffold, or an aptamer.

The anti-VEGF entity may act by binding to one or more VEGFs and/or one or more VEGF receptors.

The anti-VEGF entity may be capable of reducing a pro-proliferative and/or pro-angiogenic activity of VEGF.

By way of example, the anti-VEGF entity may be capable of reducing the proliferation of a VEGF dependent cell line. Suitable cell lines include, but are not limited to endothelial cells such as Human umbilical vein endothelial cells (HUVEC) and the Ba/F3-VEGFR2 cell line described by Wentink *et al.* (Br J Cancer; 2016; 115(8); 940-948).

The anti-VEGF entity may reduce the proliferation of a VEGF dependent cell line by at least 1-fold (suitably, at least 1.5-fold, at least 2-fold, at least 2.5-fold, at least 3-fold, at least 4-fold or at least 5-fold) relative to the proliferation in the absence of anti-VEGF entity. The anti-VEGF entity may reduce the proliferation of a VEGF dependent cell line by at least 10% (suitably, at least 20%, at least 30%, at least 40%, at least 50%, at least 100%, at least 150% or at least 200%) relative to the proliferation in the absence of the anti-VEGF entity.

The anti-VEGF entity may be selected from aflibercept, ranibizumab, bevacizumab and pegaptanib or a fragment or variant thereof.

The anti-VEGF entity may be selected from aflibercept, ranibizumab and pegaptanib or a fragment or variant thereof.

- 5 The anti-VEGF entity may be selected from aflibercept and ranibizumab or a fragment or variant thereof.

The anti-VEGF entity may be aflibercept or a fragment or variant thereof.

The anti-VEGF entity may be ranibizumab or a fragment or variant thereof.

The anti-VEGF entity may be bevacizumab or a fragment or variant thereof.

- 10 The anti-VEGF entity may be pegaptanib or a fragment or variant thereof.

The anti-VEGF entity may be aflibercept. Aflibercept binds to circulating VEGFs and acts as a "VEGF trap". It thereby inhibits the activity of the VEGF subtypes VEGF-A and VEGF-B, as well as PGF. Aflibercept is a recombinant protein composed of the binding domains of VEGFR1 and VEGFR2 fused with the Fc region of human immunoglobulin gamma 1 (IgG1).

- 15 Structurally, aflibercept is a dimeric glycoprotein with a protein molecular weight of 96.9 kDa.

An illustrative aflibercept amino acid sequence is shown as SEQ ID NO: 1.

SEQ ID NO: 1

20 SDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWD
 RKGFIISNATYKEIGLLTCEATVNGHLYKTNLYLTHRQTNTIIDVLSPSHGIELSVGEKL
 VLNCTARTELVNIGIDFNWEYPSSKHQHKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQ
 GLYTCAASSGLMTKKNSTFVRVHEKDKHTCPCPCAPPELLGGPSVFLFPPKPKDTLMISR
 TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
 GKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS
 25 DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNH
 YTQKSLSLSPG

The aflibercept amino acid sequence may comprise a sequence shown as SEQ ID NO: 1.

The variant may have 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: 1. Preferably, a fragment or variant substantially retains a functional activity of the protein shown as SEQ ID NO: 1.

- 30 The aflibercept amino acid sequence may be encoded by a polynucleotide sequence shown as SEQ ID NO: 2-11.

SEQ ID NO: 2

AGTGATACAGGTAGACCTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATACACATGACTGAAGGAAGGGAG
 CTCGTCAATCCCTGCCGGTTACGTACCTAACATCACTGTTACTTTAAAAAGTTTCCACTTGACACTTTGATC
 CCTGATGGAAAACGCATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGG
 5 CTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAACTATCTCACACATCGACAAACCAATACA
 ATCATAGATGTCGTTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTGGAGAAAAGCTTGTCTTAAATTGTACA
 GCAAGAACTGAACTAAATGTGGGGATTGACTTCAACTGGGAATACCCTTCTTCGAAGCATCAGCATAAGAAACTT
 GTAAACCGAGACCTAAAAACCCAGTCTGGGAGTGAGATGAAGAAATTTTGGAGCACCTTAACTATAGATGGTGTA
 ACCCGGAGTGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACATTTGTC
 10 AGGGTCCATGAAAAAGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTC
 TTCCTCTTCCCCCAAACCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGAC
 GTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAA
 CCGCGGGAGGAGCAGTACACAGCACGTACCGTGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAAT
 GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAA
 GGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCATCCCGGATGAGCTGACCAAGAACCAGGTGAGCCTG
 15 ACCTGCCTGCTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC
 TACAAGACCACGCCTCCCGTGTCTGAGTCCGACGGCTCCTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGC
 AGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGC
 CTCTCCCTGTCTCCGGTTGA

In some embodiments, the nucleotide sequences encoding aflibercept (or any other anti-
 20 VEGF entity) used in the 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
 25 codons for which the corresponding tRNAs are known to be rare in the particular cell type.
 Thus, an additional degree of translational control is available.

Illustrative codon optimized nucleotide sequences encoding aflibercept are shown as SEQ ID
 NO: 3-11.

SEQ ID NO: 3

30 TCTGATACCGGCAGACCTTTCGTGGAATGTACAGCGAGATCCCCGAGATCATCCACATGACCGAGGGCAGAGAG
 CTGGTCATCCCTGCAGAGTGACAAGCCCCAACATCACCGTGACTCTGAAGAAGTTCCTCTGGACACACTGATC
 CCGGACGGCAAGAGAATCATCTGGGACAGCCGGAAGGGCTTCATCATCAGCAACGCCACCTACAAAGAGATCGGC
 CTGCTGACCTGTGAAGCCACCGTGAATGGCCACCTGTACAAGACCAACTACCTGACACACAGACAGACCAACACC
 35 ATCATCGACGTGGTGTGAGCCCTAGCCACGGCATTGAACTGTCTGTGGGCGAGAAGCTGGTGTGAAGTGTACC
 GCCAGAACCAGCTGAACGTGGGCATCGACTTCAACTGGGAGTACCCAGCAGCAAGCACCAGCACAAGAACTG
 GTCAACCGGGACCTGAAAACCCAGAGCGGCAGCGAGATGAAGAAATTCCTGAGCACCTTGACCATCGACGGCGTG
 ACCAGATCTGACCAGGGCTGTACACATGTGCCGCCAGCTCTGGCCTGATGACCAAGAAAAACAGCACCTTCGTG
 CGGGTGCACGAGAAGGACAAGACCCACACCTGTCTCCATGTCTGCTCCAGAAGTGTGCGGCGGACCTTCCGTG
 40 TTCTGTTTCTCCAAAGCCTAAGGACACCCTGATGATCAGCAGAACCCTGAAGTGACCTGCGTGGTGGTGGAT
 GTGTCCACGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGACAACGCCAAGACCAAG
 CCTAGAGAGGAACAGTACAATAGCACCTACAGAGTGGTGTCCGTGCTGACCGTGTGACCCAGGATTGGCTGAAC
 GGCAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCTATCGAGAAAACCATCTCCAAGGCCAAG
 45 GGCCAGCCTAGGGAACCCAGGTTTACACACTGCCTCCAAGCAGGGACGAGCTGACAAAGAACCAGGTGTCCCTG
 ACCTGCCTGGTCAAGGGCTTCTACCTTCCGATATCGCCGTGGAATGGGAGAGCAATGGCCAGCCTGAGAACAAC
 TACAAGACAACCCCTCCTGTGCTGGACAGCGACGGCTATTCTTCTGTACAGCAAGCTGACAGTGGACAAGAGC
 AGATGGCAGCAGGGCAACGTGTTTACGTGCAGCGTGATGCACAGGCCCTGCACAACCACTACACCCAGAAGTCC
 CTGAGCCTGTCTCCTGGCTAA

SEQ ID NO: 4

TCTGATACCGGCAGACCCTTCGTGGAAATGTACAGCGAGATCCCCGAGATCATTCACATGACCGAGGGCAGAGAG
CTGGTCATCCCCTGCCGAGTGACAAGCCCCAACATCACCGTGACTCTGAAGAAATTCCTCTGGACACACTGATC
CCCGACGGCAAGAGAATCATTTGGGACAGCCGGAAGGGCTTCATCATTAGCAACGCCACCTACAAAGAGATCGGC
CTGCTCACCTGTGAAGCCACCGTGAATGGCCACCTGTACAAGACCAACTACCTGACACACAGACAGACCAACACC
5 ATCATTGACGTGGTCTGAGCCCTAGCCACGGCATTGAAGTGTCTGTGGGCGAGAAGCTGGTGTGAAGTGTACC
GCCAGAACCAGAGCTGAACGTGGGCATCGACTTCAACTGGGAGTACCCAGCTCCAAGCACCAGCACAGAAACTG
GTCAACCGGGACCTGAAAACCCAGAGCGGCAGCGAGATGAAGAAATTCCTGAGCACCTGACCATCGACGGCGTG
ACCAGATCTGACCAGGGCCTGTACACATGTGCCGCTAGCTCTGGCCTGATGACCAAGAAAAACAGCACCTTCGTG
CGGGTGACAGAGAAGGACAAGACCCACACCTGTCTCCATGTCTGCTCCCGAACTGCTCGGCGGACCTTCCTGTG
10 TTCCTGTTTCTCCAAAGCCTAAGGACACCCTGATGATCAGCAGAACCCTGAAGTGACCTGCGTGGTCTGGAT
GTGTCCCACGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGACACAACGCCAAGACCAAG
CCTAGAGAGGAACAGTACAAATAGCACCTACAGAGTGGTCTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAAC
GGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCTATCGAGAAAACCATCTCCAAGGCCAAG
GGCCAGCCTAGGGAACCCAGGTTTACACACTGCCTCCAAGCAGGGACGAGCTGACAAAGAACCAGGTGTCCCTG
15 ACCTGCCTGGTCAAGGGCTTCTACCCCTCCGATATCGCCGTGGAATGGGAGAGCAATGGCCAGCTGAGAACAAT
TACAAGACAACCCCTCCCGTGTGACAGCGACGGCTCATTCTTTCTGTACTCCAAGCTGACAGTGGACAAGAGC
AGATGGCAGCAAGGCAACGTGTTTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCC
CTGAGCCTGTCTCCTGGCTAA

SEQ ID NO: 5

AGCGACACCGGCAGACCCTTCGTGGAGATGTACTCCGAGATCCCTGAGATCATCCACATGACCGAGGGCAGGGAG
CTGGTCATCCCATGCCGCTGACATCCCCAACATCACCGTGACTCTGAAGAAGTTCCTCTGGACACCCTGATC
CCAGATGGCAAGCGGATCATCTGGGACTCTAGAAAGGGCTTTATCATCAGCAATGCCACATATAAGGAGATCGGC
CTGCTGACCTGCGAGGGCCACAGTGAACGGCCACCTGTACAAGACCAATTATCTGACACACAGACAGACCAACACA
ATCATCGATGTGGTGTCTCCCATCTCACGGCATCGAGCTGAGCGTGGGCGAGAAGCTGGTGTGAATTGTACC
25 GCCAGGACAGAGCTGAACGTGGGCATCGACTTCAATTGGGAGTACCCAGCTCCAAGCACCAGCACAGAAGCTG
GTGAACAGGGATCTGAAGACCCAGAGCGGCTCCGAGATGAAGAAGTTTCTGTCTACCCTGACAATCGACGGAGTG
ACCCGCGAGCGATCAGGGACTGTATACATGCGCCGCTCTAGCGGCCTGATGACCAAGAAGAAGCAGCACCTTCGTG
CGGGTGACAGAGAAGGACAAGACCCACACATGCCACCTTGTCCAGCACCAGAGCTGCTGGGAGGACCAAGCGTG
TTCCTGTTTCCACCCAAGCCCAAGGATACCCTGATGATCTCTCGCACCCCGAGGTGACATGCGTGGTGGTGGAC
30 GTGAGCCACGAGGACCCGAGGTGAAGTTTAACTGGTACGTGGACGGCGTGAGGTGCACAATGCCAAGACCAAG
CCTCGGGAGGAGCAGTACAACAGCACCTATAGAGTGGTGTCCGTGCTGACAGTGTGCACCAGGATTGGCTGAAC
GGCAAGGAGTATAAGTGCAAGGTGTCCAATAAGGCCCTGCCTGCCCAATCGAGAAGACCATCTTAAGGCAAAG
GGACAGCCCAGGGAGCCTCAGGTGTACACACTGCCTCCATCCCGCGACGAGCTGACCAAGAACCAGGTGTCTCTG
ACATGTCTGGTGAAGGGCTTCTATCTTAGCGACATCGCCGTGGAGTGGGAGTCCAATGGCCAGCCAGAGAACAAT
35 TACAAGACCACACCCCTGTGCTGGACTCCGATGGCTCTTTCTTTCTGTATTCCAAGCTGACCGTGGATAAGTCT
CGGTGGCAGCAGGGCAACGTGTTTTCTTGTAGCGTGATGCACGAGGCCCTGCACAATCACTACACACAGAAGTCC
CTGTCTCTGAGCCCCGGCTGA

SEQ ID NO: 6

AGCGACACCGGCAGACCCTTCGTGGAGATGTACTCCGAGATCCCTGAGATCATTCACATGACCGAGGGCAGGGAG
CTGGTCATCCCATGCCGCTGACATCCCCAACATCACCGTGACTCTGAAGAAGTTCCTCTGGACACCCTGATC
CCAGATGGCAAGCGGATCATTTGGGACTCTAGAAAGGGCTTTATCATTAGCAATGCCACATATAAGGAGATCGGC
CTGCTCACCTGCGAGGGCCACAGTGAACGGCCACCTGTACAAGACCAATTATCTGACACACAGACAGACCAACACA
ATCATTGATGTGGTCTGTCCCATCTCACGGCATCGAGCTGAGCGTGGGCGAGAAGCTGGTGTGAATTGTACC
45 GCCAGGACAGAGCTGAACGTGGGCATCGACTTCAATTGGGAGTACCCAGCTCCAAGCACCAGCACAGAAGAACTG
GTGAACAGGGATCTGAAGACCCAGAGCGGCTCCGAGATGAAGAAATTTCTGTCTACCCTGACAATCGACGGAGTG
ACCCGCGAGCGATCAGGGACTGTATACATGCGCCGCTTCTAGCGGCCTGATGACCAAGAAAAACAGCACCTTCGTG
CGGGTGACAGAGAAGGACAAGACCCACACATGCCACCTTGTCCAGCACCAGAGCTGCTCGGAGGGCCCAAGCGTG
TTCCTGTTTCCACCCAAGCCCAAGGATACCCTGATGATCTCTCGCACCCCGAGGTGACATGCGTGGTCTGGAC
GTGAGCCACGAGGACCCGAGGTGAAGTTTAACTGGTATGTGGACGGCGTGAGGTGCACAATGCCAAGACCAAG
50 CCTCGGGAGGAACAGTACAACAGCACCTATAGAGTGGTCTCCGTGCTGACAGTGTGCACCAGGATTGGCTGAAC
GGCAAGGAGTATAAGTGCAAGGTGTCCAATAAGGCCCTGCCTGCCCAATCGAGAAGACCATCTTAAGGCAAAG
GGACAGCCCAGGGAGCCTCAGGTGTACACACTGCCTCCATCCCGCGACGAGCTGACCAAGAACCAGGTGTCTCTG
ACATGTCTGGTGAAGGGCTTCTATCTTAGCGACATCGCCGTGGAGTGGGAGTCCAATGGCCAGCCAGAGAACAAT
TACAAGACCACACCCCTGTGCTGGACTCCGATGGCTCTTTCTTTCTGTATTCCAAGCTGACCGTGGATAAGTCT
55 CGGTGGCAGCAAGGCAACGTGTTTTCTTGTTCGGTGTGATGCACGAGGCCCTGCACAATCACTACACACAGAAGTCC
CTGTCTCTGAGCCCCGGCTGA

SEQ ID NO: 7

TCCGACACCGGCAGACCCTTCGTGGAGATGTACAGCGAGATCCCCGAGATCATTCACATGACCGAGGGCAGAGAG
CTCGTGATCCCTTG TAGAGTGACCTCCCCAACATCACCGTGACACTGAAGAAGTTTCCTCTGGATACCCCTCATC
CCCGACGGCAAGAGGATTATTTGGGACTCTAGAAAGGGCTTCATCATTAGCAACGCCACCTACAAGGAGATCGGA
5 CTGCTGACATGCGAGGCCACCGTGAACGGCCACCTCTACAAGACCACTATCTGACCCATAGACAGACCAATACC
ATCATCGACGTGGTGCTCTCCCCTAGCCACGGAATCGAGCTGAGCGTGGGCGAGAACTGGTGCTCAATTGCACC
GCTAGAACAGAGCTCAACGTGGGCATCGACTTCAACTGGGAATACCCCTCCTCCAAGCACCAACACAAGAAGCTC
GTGAATAGAGATCTGAAGACCCAAAGCGGCAGCGAGATGAAGAAGTTTCTGAGCACACTGACAATTGACGGCGTC
ACAAGAAGCGACCAAGGCCTCTATACATGCGCGCCAGCAGCGGACTGATGACCAAGAAGAACAGCACCTTCGTG
10 AGAGTGACGAGAAAGATAAGACCCACACATGTCCCCCTTGCCCCGCCCCGAGCTGCTGGGAGGACCTAGCGTC
TTTCTGTTCCCTCCTAAGCCCAAGGACACACTGATGATCTCCAGAACCCCCGAGGTGACATGCGTGGTCTGGAC
GTGTCCCACGAGGACCCCGAGGTGAAGTTCAATTGGTACGTGACGGCGTGGAGGTGCACAATGCTAAGACCAAG
CCCAGAGAGGAGCAGTACAACCTCCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTCCATCAAGACTGGCTCAAC
GGCAAGGAGTAGAAGTGCAAGGTGAGCAACAAGGCCCTCCCCGCCCTATCGAGAAGACCATTTCGAAGGCCAAG
15 GGCCAGCCTAGAGAGCCCCAAGTGTATACACTGCCTCCCAGCAGAGACGAGCTCACCAAAAACCAAGTGTCCCTC
ACATGTCTGGTCAAAGGCTTCTACCCAGCGATATCGCCGTGGAGTGGGAAAGCAACGGCCAGCCCGAGAACAAC
TACAAGACAACCCCTCCCGTGTCTGACAGCGACGGAAGCTTCTTTCTGTACAGCAAGCTGACCGTGGACAAGTCT
AGATGGCAGCAAGGCAATGTCTTTAGCTGCAGCGTGATGCACGAGGCTCTGCACAATCACTACACCCAGAAGAGC
CTCTCTCTGAGCCCCGGCTGA

20 SEQ ID NO: 8

TCCGACACCGGCAGACCCTTCGTGGAGATGTACAGCGAGATCCCCGAGATCATTCACATGACCGAGGGCAGAGAG
CTCGTGATCCCTTGTCGAGTGACCTCCCCAACATCACCGTGACACTGAAGAAATTTTCCTCTGGATACCCCTCATC
CCCGACGGCAAGAGGATTATCTGGGACTCTAGAAAGGGCTTCATCATTAGCAACGCCACCTACAAGGAGATCGGA
CTGCTTACATGCGAGGCCACCGTGAACGGCCACCTCTACAAGACCACTATCTGACCCATAGACAGACCAATACC
25 ATCATTGACGTGGTTCTCTCCCCTTCTACGGAATCGAGCTGAGCGTGGGCGAGAACTGGTGCTCAATTGCACC
GCTAGAACAGAGCTCAACGTGGGCATCGACTTCAACTGGGAATACCCCTCCTCTAAGCACCAACACAAGAACTC
GTGAATAGAGATCTGAAGACCCAAAGCGGCAGCGAGATGAAGAATTTCTGAGCACACTGACAATTGACGGCGTC
ACAAGAAGCGACCAAGGCCTCTATACATGCGCGCTAGCAGTGGACTGATGACCAAGAAAAACAGCACCTTCGTG
AGAGTGACGAGAAAGATAAGACCCACACATGTCCCCCTTGCCCCGCCCCGAGGTGCTTGGAGGTCTTAGCGTC
30 TTTCTGTTCCCTCCAAAGCCCAAGGACACACTGATGATCTCCAGAACCCCCGAGGTGACATGCGTGGTCTGGAC
GTGTCCCACGAGGACCCCGAGGTGAAGTTCAATTGGTATGTTGACGGCGTGGAGGTGCACAATGCTAAGACCAAG
CCAGAGAGGAACAGTACAACCTCCACCTACAGAGTGGTTTCCGTGCTGACCGTGCTCCATCAAGACTGGCTCAAC
GGCAAGGAGTACAAGTGCAAAGTGAGCAACAAGGCCCTCCCCGCCCTATCGAGAAGACCATTTCGAAGGCCAAG
GGCCAGCCTAGAGAGCCCCAAGTGTATACACTGCCTCCCAGCAGAGACGAGCTCACCAAAAACCAAGTGTCCCTC
35 ACATGTCTGGTCAAAGGCTTCTACCCAGCGATATCGCCGTGGAGTGGGAAAGCAACGGCCAGCCCGAGAACAAT
TACAAGACAACCCCTCCCGTGTCTGACAGCGACGGAAGCTTCTTTCTGTACTCCAAGCTGACCGTGGACAAGTCT
AGATGGCAGCAAGGCAATGTCTTTAGCTGCAGCGTGATGCACGAGGCTCTGCACAATCACTACACCCAGAAGAGC
CTCTCTCTGAGCCCCGGCTGA

SEQ ID NO: 9

TCCGACACCGGGAGACCGTTTG TAGAAATGTACTCTGAAATTCAGAAATTATTCACATGACTGAAGGACGAGAA
CTCGTAATCCCGTGCCGGGTAACATCACCGAACATTACCGTTACTCTCAAGAAGTTCCCGTTGGACACTCTGATT
CCAGACGGCAAGCGGATTATATGGGATTACAGAAAAGGCTTTATCATCAGCAACGCTACATATAAGGAGATAGGC
CTCCTCACCTGCGAGGCCACAGTGAACGGACACCTTTACAAGACAACTACCTCACGCATCGCCAGACTAACACT
ATTATAGACGTTGTACTCAGCCCTTCCACGGAATAGAGCTCTCTGTTGGGGAAAAGCTTGTCTGAACTGCACG
45 GCACGAACCGAACTCAACGTTGGTATTGATTTCAACTGGGAATACCCCTCTTCAAAGCACCAACATAAGAAGCTC
GTGAACAGGGACCTGAAGACGCGAGTGGATCCGAAATGAAGAATTTCTTTCAACTCTCACATCGACGGCGTT
ACCAGGAGCGATCAGGGGCTTTATACTTGCGCGGCTTCTTCTGGACTCATGACAAAGAAAAACAGTACTTTCGTT
CGCGTCCATGAGAAAGATAAGACCCATACGTGCCACCTTGTCCAGCTCCGGAACCTTTGGGCGGACCCAGCGTA
TTTCTTTTCTCCCAAGCCGAAAGACACACTGATGATATCAAGAACTCCTGAAGTTACATGTGTTGTTGTTGAC
50 GTTAGTCATGAAGACCTGAGGTCAAGTTCAACTGGTATGTTGATGGAGTAGAGGTTTACATAACCGGAAGACGAAG
CCACGAGAAGAACAGTACAACAGCACGTATAGGGTGGTCAGTGTACTTACGGTACTCCACCAAGATTGGCTTAAC
GGTAAGGAATACAAGTGTAAAGTATCTAATAAGGCATTGCCGGCTCCGATTGAAAAGACAATCTTAAGGCAAAA
GGCCAGCCGCGGAGCCGCAAGTATATACATTGCCTCCCTCAAGAGATGAGCTTACCAAGAACCAGGTGAGCCTG
ACATGCCTTGTCAAAGGATTCTACCCTAGCGACATAGCCGTGCAATGGGAATCAAACGGTCAACCAGAGAACAAT
55 TATAAAACCAACCCCCCGGTGCTTGATAGTGATGGCTCCTTTTTCTGTATTCTAAGTTGACGGTGGATAAGAGT

CGGTGGCAACAGGGGAATGTATTTAGTTGCAGTGTGATGCACGAGGCTCTTCATAACCACTACACACAAAAATCT
TTGAGCTTGTCTCCAGGTTGA

SEQ ID NO: 10

5 TCCGACACCGGGAGACCGTTTGTAGAAATGTA CTCTGAAATTCCAGAAATTATCCACATGACTGAAGGACGAGAA
CTCGTAATCCCGTGCCGGGTAACATCACCGAACATTACCGTTACTCTCAAGAAATTCCTGTTGGACACTCTGATT
CCAGACGGCAAGCGGATTATATGGGATTACGAAAAGGCTTTATCATTAGCAACGCTACATATAAGGAGATAGGC
CTCCTGACCTGCGAGGCCACAGTGAACGAGACACCTTTACAAGACAACTACCTCACGCATCGCCAGACTAACCT
ATTATAGACGTTGTA CTCTCAGCCCTTCCCACGGAATAGAGCTCTCTGTTGGGGAAAAGCTTGTCTGAACTGCACG
GCACGAACCGAACTCAACGTTGGTATTGATTTCAACTGGGAATACCCCTCTTCAAAGCACCAACATAAGAACTC
10 GTGAACAGGGACCTGAAGACGCAGAGTGGATCCGAAATGAAGAAATTTCTTTCAACTCTCACAACTCGACGGCGTT
ACCAGGAGCGATCAGGGGCTTTATACTTGCGCGGCTTCTTCCGGACTCATGACAAAAGAAAAACAGTACTTTTCGTT
CGCGTCCATGAGAAAGATAAGACCCATACGTGCCACCTTGTCAGCTCCGGAACCTTTGGGCGGACCCAGCGTA
TTTCTTTTTCTCTCCCAAGCCGAAAGACACACTGATGATATCAAGAACTCCTGAAGTTACATGTGTTGTGTTGAC
GTCAGTCATGAAGACCTGAGGTCAAGTTCAACTGGTATGTTGATGGAGTAGAGGTTACATAACGCGAAGACGAAG
15 CCACGAGAAGAGCAGTACACAGCAGCTATAGGGTGGTCAGTGTACTTACGGTACTCCACCAAGATTGGCTTAAC
GGAAAGGAATACAAGTGTAAAGTATCTAATAAGGCATTGCCGGCTCCGATTGAAAAGACAATCTCTAAGGCAAAA
GGCCAGCCGCGGAGCCGCAAGTATATACATTGCCCTCCCTCAAGAGATGAGCTTACCAAGAACCAGGTTTCTCTG
ACATGCCTTGTCAAAGGATTCTACCCTAGCGACATAGCCGTGGAATGGGAATCAAACGGTCAACGAGAGAACAAT
TATAAAACCACACCCCGGTGCTTGATAGTGTGATGGCTCTTTTCTGTATTCTAAGTTGACGGTGGATAAGAGT
20 CGGTGGCAACAGGGGAATGTATTTAGTTGCAGTGTGATGCACGAGGCTCTTCATAACCACTACACACAAAAATCT
TTGAGCTTGTCTCCAGGTTGA

SEQ ID NO: 11

AGCGACACCGGCCGCCCTTTCGTGGAGATGTACAGCGAGATCCCCGAGATCATCCACATGACCGAGGG
CCGCGAGCTGGTGATCCCCGTGCCGCTGACCAGCCCCAACATCACCGTGACCTGAAGAAGTTCCCCC
25 TGGACACCTGATCCCCGACGGCAAGCGCATCATCTGGGACAGCCGCAAGGGCTTCATCATCAGCAAC
GCCACCTACAAGGAGATCGGCCCTGCTGACCTGCGAGGCCACCGTGAACGGCCACCTGTACAAGACCAA
CTACCTGACCCACCGCCAGACCAACACCATCATCGACGTGGTGCTGAGCCCCAGCCACGGCATCGAGC
TGAGCGTGGGCGAGAAGCTGGTGCTGAACTGCACCGCCCGCACCAGCTGAACGTGGGCATCGACTTC
AACTGGGAGTACCCAGCAGCAAGCACCAGCACAAGAAGCTGGTGAACCGCGACCTGAAGACCCAGAG
30 CGGCAGCGAGATGAAGAAGTTCTTGAGCACCCTGACCATCGACGGCGTGACCCGACGAGCCAGGGCC
TGTAACCTGCGCCGCCAGCAGCGGCCTGATGACCAAGAAGAACAGCACCTTCGTGCGCGTGCACGAG
AAGGACAAGACCCACACCTGCCCCCCTGCCCCGCCCCGAGCTGCTGGGCGGCCCCAGCGTGTTCTCT
GTTCCCCCACAAGCCCAAGGACACCCTGATGATCAGCCGCACCCCGAGGTGACCTGCGTGTTGGTGG
ACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGAGGTTGCACAACGCC
35 AAGACCAAGCCCCGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGAGCGTGCTGACCGTGCTGCA
CCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAAGGCCCTGCCCCGCCCTCG
AGAAGACCATCAGCAAGGCCAAGGCCAGCCCCGCGAGCCCCAGGTGTACACCCTGCCCCCAGCCGC
GACGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCAGCGACATCGC
CGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACCTACAAGACCACCCCCCGCTGCTGGACAGCG
40 ACGGCAGCTTCTTCTGTACAGCAAGCTGACCGTGGACAAGAGCCGCTGGCAGCAGGGCAACGTGTTT
AGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCCG
CTAA

Preferably, the polynucleotide encoding the aflibercept polypeptide is SEQ ID NO: 11 or a variant thereof.

45 In some embodiments, the nucleotide sequence encoding aflibercept has at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to any of SEQ ID NO: 2-11, preferably SEQ ID NO: 11. Preferably, the protein encoded by the

nucleotide sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 1.

Ranibizumab is a monoclonal antibody fragment (Fab) against VEGFA. Suitably, the ranibizumab polypeptide may comprise heavy and light chain variable sequences shown as
 5 SEQ ID NO: 12 and 13, respectively. Complementarity determining regions (CDRs) are shown underlined in each of the sequences.

SEQ ID NO: 12

10 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTS
 KSTAYLQMNLSRAEDTAVYYCAKYPYYGTSHWYFDVWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
 LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS
 CDKTHL

SEQ ID NO: 13

15 DIQLTQSPSSLSASVGDRTTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSLHSGVPSRFSGSGSGTDFTLTI
 SSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
 DNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

The ranibizumab heavy and light chain variable sequences may comprise polypeptides shown as SEQ ID NO: 12 or 13. The variants may have 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 or 13. Preferably, the fragment or variant substantially retains a functional activity of the heavy
 20 and light chain variable sequences shown as SEQ ID NO: 12 and 13.

The ranibizumab heavy and light chain polypeptides may be encoded by any suitable polynucleotide sequences. The polynucleotide sequences encoding the heavy and light chains may be operably linked in an isolated polynucleotide. For example, the polynucleotide sequences encoding the heavy and light chains may be operably linked to the same promoter
 25 and be separated from each other by an internal ribosomal entry site (IRES) or a polynucleotide sequence encoding a self-cleaving polypeptide.

Examples of polynucleotide sequences which encode ranibizumab heavy and light chain polypeptides; respectively, are shown as SEQ ID NO: 14 and 15.

SEQ ID NO: 14

30 GAGGTGCAGCTGGTGGAGAGCGGCGGGCGGCTGGTGCAGCCCGGCGGCAGCCTGCGCCTGAGCTGCGCGCCAGC
 GGCTACGACTTACCCACTACGGCATGAACTGGGTGCCAGGCCCGGCAAGGGCCTGGAGTGGGTGGGCTGG
 ATCAACACCTACACCGGCGAGCCACCTACGCCGCCGACTTCAAGCGCCGCTTCACCTTACAGCTGGACACCAGC
 AAGAGCACC GCCTACCTGCAGATGAACAGCCTGCGCGCCGAGGACACCGCCGTGTACTACTGCGCCAAGTACCCC
 35 TACTACTACGGCACCAGCCACTGGTACTTCGACGTGTGGGGCCAGGGCACCTGGTGACCGTGAGCAGCGCCAGC
 ACCAAGGGCCCCAGCGTGTCCCCCTGGCCCCAGCAGCAAGAGCACCAGCGGCGGCACCGCCGCCCTGGGCTGC
 CTGGTGAAGGACTACTTCCCCGAGCCCGTGACCGTGAGCTGGAACAGCGGCGCCCTGACCAGCGGCGTGACACCC
 TTCCCCGCGCTGCTGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGC

ACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCCAAGAGC
TGCGACAAGACCCACCTG

SEQ ID NO: 15

5 GACATCCAGCTGACCCAGAGCCCCAGCAGCCTGAGCGCCAGCGTGGGCGACCGCGTGACCATCACCTGCAGCGCC
AGCCAGGACATCAGCAACTACCTGAACTGGTACCAGCAGAAGCCCGGCAAGGCCCAAGGTGCTGATCTACTTC
ACCAGCAGCCTGCACAGCGGCGTGCCACGCCGCTTCAGCGGCAGCGGCAGCGGCACCGACTTCACCTGACCATC
AGCAGCCTGCAGCCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACAGCACCGTGCCCTGGACCTTCGGCCAG
GGCACCAAGGTGGAGATCAAGCGCACCGTGGCCGCCCCAGCGTGTTTCATCTTCCCCCAGCGACGAGCAGCTG
AAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACCTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTG
10 GACAACGCCCTGCAGAGCGGCAACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCTG
AGCAGCACCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGC
CTGAGCAGCCCCGTGACCAAGAGCTTCAACCGCGGCGAGTGC

Examples of isolated polynucleotides, and AAV vectors, encoding ranibizumab are provided by WO2019/164854, for example.

- 15 Bevacizumab is a monoclonal antibody against VEGFA. Suitably, the bevacizumab polypeptide sequence may comprise heavy and light chain sequences shown as SEQ ID NO: 16 and 17, respectively. Complementarity determining regions (CDRs) are shown underlined in each of the sequences.

SEQ ID NO: 16

20 EVQLVESGGGLVQPGGSLRLSCAASGYTFNTNYGMNWRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTS
KSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV
25 KGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSLSLS
PGK

SEQ ID NO: 17

DIQMTQSPSSLSASVGDRTITCSASQDISNYLNWYQQKPGKAPKVLIIYFTSSSLHSGVPSRFSGSGSGTDFTLTIT
SSLQPEDFATYYCQYSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKV
30 DNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

- The bevacizumab heavy and light chain sequences may comprise polypeptides shown as SEQ ID NO: 16 or 17. The variants may have 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: 16 or 17. Preferably, the fragment or variant substantially retains a functional activity of the heavy and light chain sequences shown as SEQ ID NO: 16 and 17.
- 35

Examples of polynucleotide sequences which encode bevacizumab heavy and light chain polypeptides; respectively, are shown as SEQ ID NO: 18 and 19.

SEQ ID NO: 18

GAGGTGCAGCTGGTGGAGAGCGGCGGCGCCTGGTGCAGCCCGGCGGCAGCCTGCGCCTGAGCTGCGCCGCCAGC
 GGCTACACCTTACCAACTACGGCATGAAGTGGGTGCGCCAGGCCCCCGCAAGGGCCTGGAGTGGGTGGGCTGG
 ATCAACACCTACACCGGCGAGCCACCTACGCCGCCGACTTCAAGCGCCGCTTACCTTACGCTGGACACCAGC
 AAGAGCACCGCTACCTGCAGATGAACAGCCTGCGCGCCGAGGACACCGCCGTGTACTACTGCGCCAAGTACCCC
 5 CACTACTACGGCAGCAGCCACTGGTACTTCGACGTGTGGGGCCAGGGCACCTGGTGACCGTGAGCAGCGCCAGC
 ACCAAGGGCCCCAGCGTGTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGCGGCACCGCCGCCCTGGGCTGC
 CTGGTGAAGGACTACTTCCCCGAGCCCGTGACCGTGAGCTGGAACAGCGCGCCCTGACCAGCGCGTGACACCC
 TTCCCCGCCGTGCTGCAGAGCAGCGCCCTGTACAGCCTGAGCAGCGTGGTGACCGTGCCCAGCAGCAGCCTGGGC
 10 ACCCAGACCTACATCTGCACGTGAACCACAAGCCAGCAACACCAAGGTGGACAGAAGGTGGAGCCCCAAGAGC
 TCGCACAAGACCCACACCTGCCCCCCCCGCCCCGAGCTGCTGGGCGGCCCCAGCGTGTTCCTGTTCCTCC
 CCCAAGCCCCAAGGACACCTGTATGATCAGCCGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAG
 GACCCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGCGAGGAG
 CAGTACAACAGCACCTACCGCGTGGTGAGCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTAC
 AAGTGCAAGGTGAGCAACAAGGCCCTGCCCGCCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGC
 15 GAGCCCCAGGTGTACACCTGCCCCCAGCCGCGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTG
 AAGGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCGAGAACAATAAGACCACC
 CCCCCGTGCTGGACAGCGACGGCAGCTTCTTCTGTACAGCAAGCTGACCGTGGACAAGAGCCGCTGGCAGCAG
 GGCAACGTGTTCACTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCAGAAGAGCCTGAGCCTGAGC
 CCGGCAAG

20 SEQ ID NO: 19

GACATCCAGATGACCCAGAGCCCCAGCAGCCTGAGCGCCAGCGTGGGCGACCGCGTGACCATCACCTGCAGCGCC
 AGCCAGGACATCAGCAACTACCTGAAGTGGTACCAGCAGAAGCCCGGCAAGGCCCCCAAGGTGCTGATCTACTTC
 ACCAGCAGCCTGCACAGCGGCGTGCCAGCCGCTTACGCGGCAGCGGCAGCGGCACCGACTTACCCCTGACCATC
 25 AGCAGCCTGCAGCCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACAGCACCGTGCCCTGGACCTTCGGCCAG
 GGCACCAAGGTGGAGATCAAGCGCACCGTGCGCGCCCCCAGCGTGTTCATCTTCCCCCCCAGCGACGAGCAGCTG
 AAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAACCTTCTACCCCGCGAGGCCAAGGTGCAGTGGAAGGTG
 GACAACGCCCTGCAGAGCGGCAACAGCCAGGAGCGGTGACCGAGCAGGACAGCAAGGACAGCACCTACAGCCTG
 AGCAGCACCTGACCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCACCAGGGC
 CTGAGCAGCCCCGTGACCAAGAGCTTCAACCGCGGCGAGTGC

30 The bevacizumab heavy and light chain polypeptides may be encoded by any suitable
 polynucleotide sequences. The polynucleotide sequences encoding the heavy and light
 chains may be operably linked in an isolated polynucleotide. For example, the polynucleotide
 sequences encoding the heavy and light chains may be operably linked to the same promoter
 and be separated from each other by an internal ribosomal entry site (IRES) or a
 35 polynucleotide sequence encoding a self-cleaving polypeptide.

Pegaptanib is a RNA aptamer specific for the VEGF₍₁₆₅₎ isoform. A RNA sequence for
 pegaptanib is shown as SEQ ID NO: 20.

SEQ ID NO: 20

GCGGAAUCAGUGAAUGCUUAUACAUCCGC

40 The pegaptanib polynucleotide sequence may comprise a sequence shown as SEQ ID NO:
 20. The variant may have 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: 20. Preferably, the fragment
 or variant substantially retains a functional activity of the polynucleotide shown as SEQ ID NO:
 20.

An example polynucleotide sequence encoding pegaptanib is shown as SEQ ID NO: 21.

SEQ ID NO: 21

CGCCTTAGTCACTTACGAATATGTAGGCG

Signal Peptide

- 5 The polynucleotide sequence encoding the anti-VEGF entity may further comprise a polynucleotide sequence encoding signal peptide to allow secretion of the anti-VEGF entity from a cell.

10 A signal peptide is a short peptide, commonly 5-30 amino acids long, typically present at the N-terminus of the majority of newly synthesized proteins that are destined towards the secretory pathway. These proteins include those that reside either inside certain organelles (for example, the endoplasmic reticulum, Golgi or endosomes), are secreted from the cell, and transmembrane proteins.

15 Signal peptides commonly contain a core sequence which is a long stretch of hydrophobic amino acids that has a tendency to form a single alpha-helix. The signal peptide may begin with a short positively charged stretch of amino acids, which helps to enforce proper topology of the polypeptide during translocation. At the end of the signal peptide there is typically a stretch of amino acids that is recognized and cleaved by signal peptidase. Signal peptidase may cleave either during or after completion of translocation to generate a free signal peptide and a mature protein. The free signal peptides are then digested by specific proteases.

- 20 The signal peptide is commonly positioned at the amino terminus of the molecule, although some carboxy-terminal signal peptides are known.

25 Signal sequences typically have a tripartite structure, consisting of a hydrophobic core region (h-region) flanked by an n- and c-region. The latter contains the signal peptidase (SPase) consensus cleavage site. Usually, signal sequences are cleaved off co-translationally, the resulting cleaved signal sequences are termed signal peptides.

Signal sequences can be detected or predicted using software techniques (see for example, <http://www.predisi.de/>). A large number of signal sequences are known, and are available in databases. For example, <http://www.signalpeptide.de> lists 2109 confirmed mammalian signal peptides in its database.

- 30 Suitably, the signal peptide may be a CFH signal peptide.

Illustrative polynucleotide sequences encoding a CFH signal peptide are shown as SEQ ID NO: 22-31.

SEQ ID NO: 22 - ATGAGACTTCTAGCAAAGATTATTTGCCTTATGTTATGGGCTATTTGTGTAGCA

SEQ ID NO: 23 - ATGAGACTGCTGGCCAAGATCATCTGCCTGATGCTGTGGGCCATCTGCGTGGCC

SEQ ID NO: 24 - ATGAGACTGCTCGCCAAGATCATTTGCCTGATGCTGTGGGCCATCTGCGTGGCC

SEQ ID NO: 25 - ATGCGGCTGCTGGCCAAGATCATCTGCCTGATGCTGTGGGCCATCTGCGTGGCA

5 SEQ ID NO: 26 - ATGCGGCTGCTCGCCAAGATCATTTGCCTGATGCTGTGGGCCATCTGCGTGGCA

SEQ ID NO: 27 - ATGAGACTGCTCGCCAAGATCATCTGTCTGATGCTGTGGGCTATCTGTGTCGCT

SEQ ID NO: 28 - ATGAGACTGCTCGCCAAGATCATTTGTCTGATGCTGTGGGCTATCTGTGTCGCT

SEQ ID NO: 29 - ATGCGCTTGCTGGCGAAGATAATCTGCCTGATGTTGTGGGCCATATGCGTAGCC

SEQ ID NO: 30 - ATGCGCTTGCTGGCGAAGATAATCTGCCTGATGTTGTGGGCCATATGCGTAGCC

10 SEQ ID NO: 31 - ATGCGCTTGCTGGCCAAGATCATCTGCCTGATGCTGTGGGCCATCTGCGTGGCC

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),
 5 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 (MCP, CD46), and glycosylphosphatidylinositol-anchored proteins, such as decay-
 10 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.

In some embodiments, the invention relates to the treatment or prevention of a complement-mediated disorder 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-
 15 activity of the complement C3b feedback cycle and/or under-activity of the C3b breakdown cycle.

The present invention comprises the use of a negative complement regulator. A negative complement regulator may refer to an entity which restricts the action of complement at one or more sites of the cascade reaction. By way of example, the negative complement regulator
 20 may be C1-inhibitor, anaphylatoxins inhibitor, C4b binding protein (C4BP), Complement Factor H (CFH), Factor H like protein 1 (FHL1), Complement Factor I (CFI), clusterin or vitronectin. The negative complement regulator may also be a surface protein which is capable of protecting cells against the attack of homologous complement; for example Complement Receptor 1 (CR1, CD35), membrane cofactor protein (CD46), and
 25 glycosylphosphatidylinositol-anchored proteins, such as decay-accelerating factor (CD55) or CD59 molecule.

Suitably, a negative complement regulator may be an inhibitor of a component of the complement activation cascade, for example an inhibitor of the complement C3b feedback cycle

30 The negative complement regulator may be selected from CFI, FHL1, CFH, CR1 and MCP or a variant or fragment thereof.

The negative complement regulator may be selected from CFI, FHL1 and CFH or a variant or fragment thereof.

The negative complement regulator may be CFI or a variant or fragment thereof.

The negative complement regulator may be FHL1 or a variant or fragment thereof.

The negative complement regulator may be CFH or a variant or fragment thereof.

The negative complement regulator may be CR1 or a variant or fragment thereof.

- 5 The negative complement regulator may be MCP or a variant or fragment thereof.

Suitably, the product may comprise one or more negative complement regulators.

Suitably, the polynucleotide may encode one or more negative complement regulators.

- 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).
- 10
15
20
25

- Thus, the subject to be treated by the invention may suffer from a complement-mediated disorder of the eye such as AMD, or may be at risk of developing such a disorder. The AMD may be wet AMD and/or dry AMD. For example, the subject may be homozygous or heterozygous susceptible for one or more SNPs associated with the complement-mediated disorder.
- 30

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);
5 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
10 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 for one or more of the rare Complement Factor I variants associated with AMD listed above).

15 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
20 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.

25 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 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
30 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.

5 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.

15 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.

20 In the foregoing section, except where obviously inapplicable, reference to Complement Factor I and C3b-inactivating and iC3b-degradation activity may be replaced with 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. 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)

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 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: 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 C3dg 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), 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 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. C3d,g 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.

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.

5 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 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.

10 Suitably, a fragment or variant 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 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
15 et al. (Biochem. J. (1982) 203: 293-298). Both haemolytic and conglutinating 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
20 Eds; Herzenberg Leonore A'Weir DM, Herzenberg Leonard A & 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
25 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: 32) of which amino acids 1 to 18 form a signal
30 sequence.

In some embodiments, the amino acid sequence of Complement Factor I is SEQ ID NO: 32. In other embodiments, the amino acid sequence of Complement Factor I is the sequence

disclosed as positions 19 to 583 of SEQ ID NO: 32. Preferably, variant or fragment substantially retains a functional activity of the protein represented by SEQ ID NO: 32

5 MKLLHVFLFLFLCFHLRFCKVITYTSQEDLVEKKCLAKKYTHLSCKVFCQPWQRCIEGTCVCKLPYQCPKNGT
AVCATNRRSFPTYCQQKSLECLHPGTFKFLNNGTCTAEGKFSVSLKHGNTDSEGIVEVKLVQDKTMFICKSS
WSMREANVACLDLGFQGGADTQRRFKLSDLINSTECLHVHCRGLETSLAECTFTKRRTMGYQDFADVVCYT
10 QKADSPMDFFQCVNGKYISQMKACDGINDCGDSDELCKACQKGKGFHCKSGVCIPSYQCNGEVDCTIGE
DEVGCAGFASVTQEETEILTADMDAERRRIKSLPKLSCGVKNRMHIRRKRIVGGKRAQLGDLWPQVAIKDA
SGITCGGIYIGGCWILTAACHCLRAKTHRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIAL
IEMKKDGNKKDCELPRIIPACVPWSPYLFQPNDCIVSGWGREKDNERVFSLQWGEVKLISNCSKFYGNRFY
EKEMECAGTYDGSIDACKGDSGGPLVCMDDANNVTYVWGVVSWGENCGKPEFPGVYTKVANYFDWISYHVGRP
FISQYNV
(SEQ ID NO: 32)

In some embodiments, the amino acid sequence of Complement Factor I is SEQ ID NO: 33, which corresponds to NCBI Accession No. NP_000195. In other embodiments, the amino acid
15 sequence of Complement Factor I is the sequence disclosed as positions 19 to 583 of SEQ ID NO: 33.

20 MKLLHVFLFLFLCFHLRFCKVITYTSQEDLVEKKCLAKKYTHLSCKVFCQPWQRCIEGTCVCKLPYQCPKNGT
AVCATNRRSFPTYCQQKSLECLHPGTFKFLNNGTCTAEGKFSVSLKHGNTDSEGIVEVKLVQDKTMFICKSS
WSMREANVACLDLGFQGGADTQRRFKLSDLINSTECLHVHCRGLETSLAECTFTKRRTMGYQDFADVVCYT
QKADSPMDFFQCVNGKYISQMKACDGINDCGDSDELCKACQKGKGFHCKSGVCIPSYQCNGEVDCTIGE
DEVGCAGFASVAQEETEILTADMDAERRRIKSLPKLSCGVKNRMHIRRKRIVGGKRAQLGDLWPQVAIKDA
SGITCGGIYIGGCWILTAACHCLRAKTHRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIAL
IEMKKDGNKKDCELPRIIPACVPWSPYLFQPNDCIVSGWGREKDNERVFSLQWGEVKLISNCSKFYGNRFY
25 EKEMECAGTYDGSIDACKGDSGGPLVCMDDANNVTYVWGVVSWGENCGKPEFPGVYTKVANYFDWISYHVGRP
FISQYNV
(SEQ ID NO: 33)

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: 34.

30 ATGAAGCTTCTTCATGTTTTCTGTTATTTCTGTGCTTCCACTTAAGGTTTTGCAAGGTCACCTTATACATCT
CAAGAGGATCTGGTGGAGAAAAAGTGCTTAGCAAAAAATATACTCACCTCTCCTGCGATAAAGTCTTCTGC
CAGCCATGGCAGAGATGCATTGAGGGCACCTGTGTTTGTAAGTACCGTATCAGTGCCCAAAGAATGGCACT
GCAGTGTGTGCAACTAACAGGAGAAGCTTCCCAACATACTGTCAACAAAAGAGTTTGGAATGTCTTCATCCA
GGGACAAAGTTTTTAAATAACGGAACATGCACAGCCGAAGGAAAGTTTAGTGTTTCTTGAAGCATGGAAT
35 ACAGATTACAGAGGGAATAGTTGAAGTAAACTTGTGGACCAAGATAAGACAATGTTTCATATGCAAAAGCAGC
TGGAGCATGAGGGAAGCCAACGTGGCTGCCTTGACCTGGGTTTCAACAAGGTGCTGATACTCAAAGAAGG
TTTAAGTTGTCTGATCTCTCTATAAATCCACTGAATGTCTACATGTGCATTGCCGAGGATTAGAGACCAGT
TTGGCTGAATGTACTTTTACTAAGAGAAGAACTATGGGTTACCAGGATTTGCTGATGTGGTTTGTATACA
CAGAAAGCAGATTCTCAATGGATGACTTCTTTAGTGTGTGAATGGGAAATACATTTCTCAGATGAAAGCC
TGTGATGGTATCAATGATTGTGGAGACCAAGTGATGAAGTGTGTTGTAAAGCATGCCAAGGCAAAGGCTTC
40 CATTGCAAAATCGGGTGTTCATTCCAAGCCAGTATCAATGCAATGGTGAGGTGGACTGCATTACAGGGGAA
GATGAAGTTGGCTGTGCAGGCTTTGCATCTGTGGCTCAAGAAGAAACAGAAATTTTGACTGCTGACATGGAT
GCAGAAAGAAGACGGATAAAATCATTATTACCTAACTATCTTGTGGAGTTAAAAACAGAAATGCACATTGCA
AGGAAACGAATTGTGGGAGGAAAGCGAGCACAAGTGGGAGACCTCCCATGGCAGGTGGCAATTAAGGATGCC
AGTGGAAATCACCTGTGGGGGAATTTATATTGGTGGCTGTTGGATTCTGACTGCTGCACATTGTCTCAGAGCC
45 AGTAAACTCATCGTTACCAAATATGGACAACAGTAGTAGACTGGATACACCCGACCTTAAACGTATAGTA
ATTGAATACGTGGATAGAATTATTTCCATGAAACTACAATGCAGGCACTTACCAAAATGACATCGCTTTG
ATTGAAATGAAAAAGACGGAAACAAAAAGATTGTGAGCTGCCTCGTTCCATCCCTGTGTCCCTGG
TCTCCTTACCTATTCCAACCTAATGATACATGCATCGTTTCTGGCTGGGGACGAGAAAAAGATAACGAAAGA

GTCTTTTCACTTCAGTGGGGTGAAGTTAACTAATAAGCAACTGCTCTAAGTTTTACGGAAATCGTTTCTAT
GAAAAAGAAATGGAATGTGCAGGTACATATGATGGTTCCATCGATGCCTGTAAAGGGGACTCTGGAGGCCCC
TTAGTCTGTATGGATGCCAACAATGTGACTTATGTCTGGGGTGTGTGAGTTGGGGGAAAACGTGTGGAAAA
CCAGAGTCCCAGGTGTTACACCAAGTGGCCAATTATTTTACTGGATTAGCTACCATGTAGGAAGGCCT
5 TTTATTTCTCAGTACAATGTATAA

(SEQ ID NO: 34)

In some embodiments, the nucleotide sequences of Complement Factor I used in the invention are codon-optimised.

A preferred nucleotide sequence encoding Complement Factor I is the nucleotide sequence
10 shown as SEQ ID NO: 35.

ATGAAACTGCTGCATGTCTTCCTCCTCTTCCTGTGCTTCCACCTCCGTTTCTGTAAAGTCACCTACACTAGC
CAGGAGGATCTGGTGGAGAAGAAATGCCTGGCCAAGAAGTATACCCACCTGAGCTGCGACAAAGTGTTCTGC
CAGCCCTGGCAACGCTGCATTGAAGGTACTTGTGTGTGCAAGCTGCCCTACCAGTGCCCCAAGAAGGCACG
15 GCCGTGTGTGCCACCACAGGAGGAGCTTCCCCACCTACTGCCAGCAGAAGAGCCTGGAAATGCCTCCACCTT
GGCACCAAGTTTCTGAACAACGGGACCTGCACAGCCGAGGGGAAATTACAGCGTCTCCCTCAAGCACGGCAAT
ACAGACTCCGAGGGCATTGTGGAAGTGAAGCTGGTGGACCAGGACAAGACCATGTTTATCTGCAAAAGCAGC
TGGTCCATGCGGGAGGCCAATGTCGCCTGCCTGGACCTGGGCTTCCAGCAGGGCGCTGATACACAGCGCCGC
20 TTTAAACTCAGTGACCTCAGCATCAACAGCACTGAGTGTCTGCACGTGCACTGCCGGGGCCTGGAGACCAGC
CTGGCTGAGTGCACCTTCACCAAGCGCAGGACCATGGGCTACCAGGATTTTGCAGATGTGGTCTGCTACACC
CAGAAGGCAGACAGCCCATGGATGACTTCTTCCAGTGTGTCAATGGCAAGTACATTTCCAGATGAAGGCT
TGTGACGGGATCAATGATTGCGGGGATCAGAGCGATGAGCTCTGCTGCAAGGCCTGCCAAGGGAAGGGCTTT
CACTGTAAGTCTGGGGTGTGCATCCCTTCTCAGTATCAGTGCAACGGAGAGGTGGACTGCATCACTGGGGAG
GACGAGGTGGGCTGTGCTGGCTTCGCCTCTGTGGCCAGGAGGAGACAGAGATCCTCACAGCTGACATGGAT
25 GCAGAGCGGCGCGCATCAAGAGTCTGCTCCCAAAGCTCTCCTGCGGCGTTAAGAATCGCATGCACATCCGG
AGGAAGCGGATCGTTGGAGGCAACGGGCTCAGCTGGGGACTTGCCGTGGCAGGTGGCCATCAAAGATGCC
TCCGGAATCACCTGTGGTGGCATCTACATCGGCGGCTGCTGGATCCTGACCGCCGCCACTGCCTTCGGGCC
AGCAAGACTCACCGTACCAGATCTGGACCACCGTGGTGGATTGGATTACCCCGACCTGAAGAGGATTGTC
ATTGAGTATGTCGACCGCATCATCTTCCATGAAACTACAATGCCGGGACGTATCAGAACGCATCGCCCTC
ATCGAGATGAAGAAGGATGGGAACAAGAAGGACTGTGAGCTGCCTCGCTCCATCCCCGCCTGTGTACCATGG
30 TCTCCGTACCTGTTCCAGCCAAATGACACATGCATCGTGAGCGGCTGGGGCCGCGAGAAAGACAACGAGAGG
GTCTTCTCCCTGCAGTGGGGTGAAGTCAAGCTGATCAGCAACTGCTCCAAGTTCTACGGCAACCGCTTCTAT
GAGAAGGAGATGGAGTGCGCCGGCACCTATGACGCGAGCATTGACGCGTGAAGGGAGACAGTGGGGGCCCC
CTGGTCTGCATGGACGCCAACAATGTGACCTACGTGTGGGAGTTGTGTCTGGGGCGAGAAGTGTGGCAAG
35 CCTGAGTTCGCGGGCGTGTACACAAAGGTGGCAAACATATTTGACTGGATCTCCTATCACGTTGGCAGGCCC
TTCATTTACAGTACAACGTA

(SEQ ID NO: 35)

A further example codon-optimised nucleotide sequence encoding Complement Factor I is
SEQ ID NO: 36.

ATGAAGCTGCTGCATGTCTTTCTGCTGTTTCTGTGCTTCCATCTGCGGTTCTGTAAAGTGACCTATACTAGC
40 CAGGAGGATCTGGTGGAGAAGAAGTGTCTGGCCAAGAAGTACACACACCTGAGCTGCGACAAGGTGTTCTGT
CAGCCTTGGCAGCGGTGCATCGAGGGCACCTGCGTGTGCAAGCTGCCTTACCAGTGCCCCAAGAAGGCACCC
GCCGTGTGCGCCACAAATCGGAGATCTTTTCCAACATATTGCCAGCAGAAGAGCCTGGAGTGTCTGCACCCC
GGCACCAAGTTCTGAACAATGGCACCTGCACAGCCGAGGGCAAGTTTTCTGTGAGCCTGAAGCACGGCAAC
45 ACAGATAGCGAGGGCATCGTGGAGGTGAAGCTGGTGGACCAGGATAAGACCATGTTTATCTGTAAGAGCTCC
TGGTCCATGAGGGAGGCAACGTGGCATGCCTGGATCTGGGATTCAGCAGGGAGCAGACACACAGAGGCGC
TTTAAGCTGTCCGACCTGTCTATCAATAGCACCGAGTGCCCTGCACGTGCACTGTAGGGGCTGGAGACATCC
CTGGCAGAGTGCACCTTCACAAAGCGGAGAACCATGGGCTACCAGGACTTTGCCGACGTGGTGTGCTATACC
CAGAAGGCCGATAGCCCATGGACGATTTCTTTTCACTGCGTGAACGGCAAGTATATCTCCAGATGAAGGCC
TGCGACGGCATCAATGACTGTGGCGATCAGTCTGACGAGCTGTGCTGTAAGGCCTGTGAGGGCAAGGGCTTC

CACTGCAAGAGCGGCGTGTGCATCCCTTCCCAGTACCAGTGCAACGGCGAGGTGGATTGTATCACAGGAGAG
 GACGAAGTGGGATGCGCAGGATTTGCATCTGTGGCACAGGAGGAGACAGAGATCCTGACAGCCGACATGGAT
 GCCGAGAGGCGCCGGATCAAGTCTCTGCTGCCTAAGCTGAGCTGTGGCGTGAAGAATCGGATGCACATCAGA
 5 AGGAAGCGCATCGTGGGAGGCAAGAGGGCACAGCTGGGCGATCTGCCATGGCAGGTGGCCATCAAGGACGCC
 TCTGGCATCACCTGCGGCGGCATCTACATCGGAGGATGTTGGATCCTGACCGCAGCACACTGCCTGAGAGCA
 AGCAAGACACACAGGTATCAGATCTGGACCACAGTGGTGGATTGGATCCACCCAGACCTGAAGAGAATCGTG
 ATCGAGTACGTGGATAGGATCATCTTTCACGAGAACTACAATGCCGGCACATATCAGAACGACATCGCCCTG
 ATCGAGATGAAGAAGGATGGCAATAAGAAGGACTGTGAGCTGCCAGATCCATCCCTGCATGCGTGCCATGG
 10 AGCCCCATCTGTTCAGCCCAACGATACCTGCATCGTGTCCGGATGGGGAAGGGAGAAGGACAATGAGCGG
 GTGTTTCTCTGCAGTGGGCGAGGTGAAGCTGATCTCCAAGTCTTAAGTTCTACGGCAATAGGTTTAT
 GAGAAGGAGATGGAGTGCGCCGGCACCTACGATGGCAGCATCGACGCCTGTAAGGGCGATTCCGGAGGACCA
 CTGGTGTGCATGGACGAAACAATGTGACATACGTGTGGGGAGTGGTGTCTGGGGAGAGAACTGCCGCAAG
 CCAGAGTTCCCCGGCGTATATACCAAGGTGGCCAATTATTTTGATTGGATTTCCTACCACGTCGGCAGGCC
 TTTATTTCCCAGTATAATGTCTAA
 15 (SEQ ID NO: 36)

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%
 or 99% identity to SEQ ID NO: 34, 35 or 36, preferably SEQ ID NO: 35. Preferably, the protein
 encoded by the nucleotide sequence substantially retains a functional activity of the protein
 20 represented by SEQ ID NO: 34, 35 or 36.

In other embodiments, the nucleotide sequence encoding Complement Factor I is SEQ ID NO:
 34, 35 or 36, preferably SEQ ID NO: 35.

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
 25 positions 55 to 1752 of SEQ ID NO: 34, 35 or 36, preferably SEQ ID NO: 35. Preferably, the
 protein encoded by the nucleotide sequence substantially retains a functional activity of the
 protein represented by SEQ ID NO: 32 or 33.

In other embodiments, the nucleotide sequence encoding Complement Factor I is positions
 55 to 1752 of SEQ ID NO: 34, 35 or 36, preferably SEQ ID NO: 35.

30 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: 32 or 33. Preferably, wherein the amino acid sequence substantially
 retains a functional activity of the protein represented by SEQ ID NO: 32 or 33.

In other embodiments, the nucleotide sequence encoding Complement Factor I encodes the
 35 amino acid sequence SEQ ID NO: 32 or 33.

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: 32 or 33. Preferably, wherein the amino acid

sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 32 or 33.

In other embodiments, the nucleotide sequence encoding Complement Factor I encodes the amino acid sequence of positions 19 to 583 of SEQ ID NO: 32 or 33.

- 5 An advantage of the invention is that Complement Factor I is particularly difficult to prepare 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
10 a site of interest, for example the eye, to enable in situ translation of the Complement Factor I polypeptide.

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
15 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
20 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
25 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 7 and CCPs 19-20 bind to GAGs and sialic acid (Schmidt et al. (2008) Journal of Immunology 181: 2610–
30 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

5 showed 91% reduction in endothelial cell proliferation and 69% attenuation of RPE atrophy. Electrophoretography 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 variant thereof

10 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 variant thereof is capable of increasing the rate of dissociation of C3 convertase and C5 convertase.

In preferred embodiments, a Complement Factor H polypeptide or a fragment or variant thereof is capable of acting as a cofactor for the Complement Factor I-mediated cleavage of

15 C3b and increasing the rate of dissociation of C3 convertase and C5 convertase.

The cofactor activity and dissociation acceleration activity of Complement Factor H, or a fragment or derivative thereof, may be determined using any suitable method known to the skilled person. For example, measurement of the cofactor activity of Complement Factor H is described in Sánchez-Corral, P., et al., 2002. The American Journal of Human Genetics,

20 71(6), pp.1285-1295 and measurement of the dissociation acceleration activity of Complement Factor H is described in Wong, E.K., et al., 2014. Journal of the American Society of Nephrology, 25(11), pp.2425-2433.

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

25 having the UniProtKB accession number P08603. This exemplified sequence is 1231 amino acids in length (disclosed as SEQ ID NO: 37) 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: 37. In other embodiments, the amino acid sequence of Complement Factor H is positions 19 to

30 1231 of SEQ ID NO: 37. Preferably, variant or fragment substantially retains a functional activity of the protein represented by SEQ ID NO: 37

MRLLAKEICMLWAI CVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPGYRSLGNVIMVCRKGEWV
ALNPLRKCQKRPCGHPGDTFPGTFTLTGGNVFEYGVKAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVV

KCLPVTAPENGKIVSSAMEPDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDVI
 NGSPISQKIIYKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNFYIPNGDYSPLRIKHRTGD
 EITYQCRNGFYPATRGNTAKCTSTGWIAPRCTLKPCDYPDIKHGGLYHENMRRPYFPVAVGKYYSYCD
 5 FETPSGSYWDHIHCTQDGWSPAVPCLRKCYFPYLENGYNQNYGRKFVQGSIDVACHPGYALPKAQTTVTCM
 ENGWSPTPRCIRVKTCSSSIDIENGFISESQTYALKEKAKYQCKLGYVTADGETSGSITCGKDGWSAQPT
 CIKSCDIPVFMNARTKNDFTWFKLNDTLDYECHDGYESNTGSTTGSIVCGYNGWSDLPICYERECELPKIDV
 HLPDRKKDQYKVGELVKFSCKPGFTIVGPNVQCYHFGLSPLDPICKEQVQSCGPPPELLNGNVKEKTKEE
 YGHSEVVEYYCNPRFLMKGNKIQCVDGEWTTLPVCIVEESTCGDIPLEHGWALSSPPYYGDSVEFNCS
 10 ESFTMI GHSRITCIHGVTQLPQCVAIDKLKKCKSSNLIILEEHLKNKKEFDHNSNIYRRCRGKEGWIHTVC
 INGRWDPEVNCMAQIQLCPPPPQIPNSHNMTTTLNRYDGEKVSVLQENYLIQEGEEITCKDGRWQSIPLC
 VEKIPCSQPPQIEHGTINSSRSSQESYAHGTKLSYTCGGFRISEENETTCYMGKWSSPPQCEGLPCKSPPE
 ISHGVAHMSDSYQYGEVITYKCFEGFGIDGPAIAKCLGEKWSHPPSCIKTCLSLPSFENAI PMGEKKD
 KAGEQVITYTCATYYKMDGASNVTICNSRWTGRPTCRDTSVNPPTVQNAIYVSRQMSKYPSEGERVRYQCRSP
 15 YEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPIDNGDITSFPLSVYAPASSVEYQCNLYQLLEGNKRITC
 RNGQWSEPPKCLHPCVISREIMENYNIALRWTAQKLYSRTGESVEFVCKRGYRLSSRSHTLRITTCWDGKLE
 YPTCAKR

(SEQ ID NO: 37)

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

20 In some embodiments, the nucleotide sequence encoding Complement Factor H is SEQ ID NO: 38.

ATGAGACTTCTAGCAAAGATTATTTGCCTTATGTTATGGGCTATTTGTGTAGCAGAAGATTGCAATGAAC
 CCTCCAAGAAAGAAATACAGAAATTCGACAGGTTCTGGTCTGACCAAACATATCCAGAAGGCACCCAGGCT
 25 ATCTATAAATGCCGCCCTGGATATAGATCTCTTGGAAATGTAATAATGGTATGCAGGAAGGGAGAATGGGTT
 GCTCTTAATCCATTAAGGAAATGTCAGAAAAGGCCCTGTGGACATCCTGGAGATACTCCTTTTGGTACTTTT
 ACCCTTACAGGAGGAAATGTGTTTGAATATGGTGTAAGGCTGTGTATACATGTAATGAGGGGTATCAATTG
 CTAGGTGAGATTAATTACCGTGAATGTGACACAGATGGATGGACCAATGATATTCCTATATGTGAAGTTGTG
 AAGTGTTTACCAAGTACAGCACCAGAGAATGAAAAATTGTCAGTAGTGCAATGGAACAGATCGGGAATAC
 30 CATTTTGGACAAGCAGTACGGTTTGTATGTAACCTCAGGCTACAAGATTGAAGGAGATGAAGAAATGCATTGT
 TCAGACGATGGTTTTTGGAGTAAAGAGAAACCAAAGTGTGTGGAATTTTCATGCAAATCCCCAGATGTTATA
 AATGGATCTCCTATATCTCAGAAGATTATTTATAAGGAGAATGAACGATTTCAATATAAATGTAACATGGGT
 TATGAATACAGTGAAAGAGGAGATGCTGTATGCACTGAATCTGGATGGCGTCCGTTGCCTTCATGTGAAGAA
 AAATCATGTGATAATCCTTATATTCCAAATGGTGACTACTCACCTTTAAGGATTAAACACAGAAGTGGAGAT
 35 GAAATCACGTACCAAGTGTAGAAATGGTTTTATCCTGCAACCCGGGGAATACAGCAAAATGCACAAGTACT
 GGCTGGATACCTGCTCCGAGATGTACCTTGAAACCTTGTGATTATCCAGACATTAACATGGAGGTCTATAT
 CATGAGAATATGCGTAGACCATACTTCCAGTAGCTGTAGGAAAATATTACTCCTATTACTGTGATGAACAT
 TTTGAGACTCCGTCAGGAAGTTACTGGGATCACATTCATTGCACACAAGATGGATGGTCGCCAGCAGTACCA
 40 TGCCTCAGAAAATGTTATTTTCTTATTTGGAAAATGGATATAATCAAAATCATGGAAGAAAGTTTGTACAG
 GGTAATCTATAGACGTTGCCTGCCATCCTGGCTACGCTCTTCCAAAAGCGCAGACCACAGTTACATGTATG
 GAGAATGGCTGGTCTCCTACTCCAGATGCATCCGTGTCAAAACATGTTCCAAATCAAGTATAGATATTGAG
 AATGGGTTTATTTCTGAATCTCAGTATACATATGCCTTAAAAGAAAAAGCGAAATATCAATGCAAACTAGGA
 45 TATGTAACAGCAGATGGTGAAACATCAGGATCAATTACATGTGGGAAAGATGGATGGTCAGCTCAACCCACG
 TGCATTAATCTTGTGATATCCCAGTATTTATGAATGCCAGAACTAAAATGACTTCACATGGTTTTAAGCTG
 AATGACACATTGGACTATGAATGCCATGATGGTTATGAAAGCAATACTGGAAGCACCCTGGTTCCATAGTG
 TGTGGTTACAATGGTTGGTCTGATTTACCCATATGTTATGAAAGAGAATGCGAACTTCTTAAATAGATGTA
 50 CACTTAGTTCCTGATCGCAAGAAAGACCAGTATAAAGTTGGAGAGGTGTTGAAATTCCTCTGCAAAACAGGA
 TTTACAATAGTTGGACCTAATTCCGTTCAAGTGCTACCACTTTGGATTGTCTCCTGACCTCCCAATATGTAAA
 GAGCAAGTACAATCATGTGGTCCACCTCCTGAACCTCCTCAATGGGAATGTTAAGGAAAAAACGAAAGAAGAA
 TATGGACACAGTGAAAGTGGTGGAATATTATTGCAATCCTAGATTTCTAATGAAGGGACCTAATAAATTCAA
 TGTGTTGATGGAGAGTGGACAACCTTTACCAAGTGTGATTGTGGAGGAGAGTACCTGTGGAGATATACCTGAA
 CTTGAACATGGCTGGGCCAGCTTTCTTCCCTCCTTATTACTATGGAGATTCAAGTGAATTCATTTGCTCA
 GAATCATTTTACAATGATTGGACACAGATCAATTACGTGTATTCTGGAGTATGGACCCAACTTCCCACTGT
 GTGGCAATAGATAAACTTAAGAAGTGCAATCATCAAATTTAATTATACTTGAGGAACATTTAAAAACCAAG
 AAGGAATTCGATCATAATTCTAACATAAGGTACAGATGTAGAGGAAAAGAGGATGGATACACACAGTCTGC

5 ATAAATGGAAGATGGGATCCAGAAGTGAAGTCAATGGCACAATAACAATTATGCCACCTCCACCTCAG
 ATTCCCAATTCTCACAATATGACAACCACACTGAATTATCGGGATGGAGAAAAAGTATCTGTTCTTTGCCAA
 GAAATTATCTAATTCAGGAAGGAGAAGAAATTACATGCAAAGATGGAAGATGGCAGTCAATACCACTCTGT
 GTTGAAAAAATTCATGTTTCAACACCTCAGATAGAACACGGAACCATTAATTCATCCAGGTCTTCACAA
 10 GAAAGTTATGCACATGGGACTAAATTGAGTTATACTTGTGAGGGTGGTTTCAGGATATCTGAAGAAAATGAA
 ACAACATGCTACATGGGAAAAATGGAGTTCTCCACCTCAGTGTGAAGGCCTTCCTTGTAATCTCCACCTGAG
 ATTTCTCATGGTGTGTAGCTCACATGTGAGACAGTTATCAGTATGGAGAAGAAGTTACGTACAAATGTTTT
 GAAGGTTTTGGAATTGATGGGCCTGCAATTGCAAAATGCTTAGGAGAAAAATGGTCTCACCTCCATCATGC
 15 ATAAAAACAGATTGTCTCAGTTTACCTAGCTTTGAAAATGCCATACCCATGGGAGAGAAGAGGATGTGTAT
 AAGGCGGGTGAGCAAGTGACTTACCTTGTGCAACATATTACAAAATGGATGGAGCCAGTAATGTAACATGC
 ATTAATAGCAGATGGACAGGAAGGCCAACATGCAGAGACACCTCCTGTGTGAATCCGCCCACAGTACAAAAT
 GCTTATATAGTGTGAGACAGATGAGTAAATATCCATCTGGTGAGAGAGTACGTTATCAATGTAGGAGCCCT
 TATGAAATGTTTGGGGATGAAGAAGTGATGTGTTTAAATGGAACTGGACGGAACCACCTCAATGCAAAGAT
 20 TCTACAGGAAAATGTGGGCCCCCTCCACCTATTGACAATGGGGACATTACTTCATTCCCCTGTGTCAGTATAT
 GCTCCAGCTTCATCAGTTGAGTACCAATGCCAGAACTTGTATCAACTTGAGGGTAACAAGCGAATAACATGT
 AGAAATGGACAATGGTCAGAACCACCAAAATGCTTACATCCGTGTGTAATATCCCAGAAATTATGGAAAAAT
 TATAACATAGCATTAAAGGTGGACAGCCAAACAGAAGCTTTATTCGAGAACAGGTGAATCAGTTGAATTTGTG
 TGTAACGGGGATATCGTCTTTCATCACGTTCTCACACATTGCGAACACATGTTGGGATGGGAACTGGAG
 TATCCAACCTTGTGCAAAAAGATAG
 (SEQ ID NO: 38)

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: 38. Preferably, wherein the protein encoded by the nucleotide sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 37.

25 In other embodiments, the nucleotide sequence encoding Complement Factor H is SEQ ID NO: 38.

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: 38. Preferably, wherein the protein encoded by the nucleotide sequence substantially
 30 retains a functional activity of the protein represented by SEQ ID NO: 37.

In other embodiments, the nucleotide sequence encoding Complement Factor H is positions 55 to 3696 of SEQ ID NO: 38.

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%
 35 identity to SEQ ID NO: 37. Preferably, wherein the amino acid sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 37.

In other embodiments, the nucleotide sequence encoding Complement Factor H encodes the amino acid sequence SEQ ID NO: 37.

In other embodiment, the nucleotide sequence encoding Complement Factor H encodes an
 40 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: 37. Preferably, wherein the amino acid sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 37.

In other embodiments, the nucleotide sequence encoding Complement Factor H encodes the amino acid sequence of positions 19 to 1231 of SEQ ID NO: 37.

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). The regulatory activity of FHL1 has been shown to be comparable to Complement Factor H (Mannes, M., et al., 2020. Frontiers in Immunology, 11; 596415).

In some embodiments, a FHL1 polypeptide or a fragment or variant thereof is capable of acting as a cofactor for the Complement Factor I-mediated cleavage of C3b. In some embodiments, a FHL1 polypeptide or a fragment or variant thereof is capable of increasing the rate of dissociation of C3 convertase and C5 convertase.

In preferred embodiments, a FHL1 polypeptide or a fragment or variant 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.

The cofactor activity and dissociation acceleration activity of a FHL1 polypeptide, or a fragment or derivative thereof, may be determined using any suitable method known to the skilled person. For example, those assays described above for Complement Factor H or any assay described in Mannes, M., et al., 2020. Frontiers in Immunology, 11; 596415.

In some embodiments, the FHL1 is human FHL1.

In some embodiments, the amino acid sequence of FHL1 is SEQ ID NO: 39. Preferably, variant or fragment substantially retains a functional activity of the protein represented by SEQ ID NO: 39. Suitably, a fragment or variant of FHL1 may retain at least 50%, 60%, 70%, 80%, 90%, 95% or 100% of the cofactor activity and/or dissociation acceleration activity of the protein represented by SEQ ID NO: 39.

MRLAKIICLMLWAICVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPGYRSLGNIIMVCRKGEWV
ALNPLRKCQKRPCGHPGDTFPGTFTLTGGNVFEYGVKAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVV
KCLPVTAPENGKIVSSAMEPDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEPKCVEISCKSPDVI
NGSPISQKIIYKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSPLRIKHRTGD
EITYQCRNGFYPATRGNTAKCTSTGWIPAPRCTLKPCDYPDIKHGGLYHENMRRPYFPVAVGKYYSYYCDEH

FETPSGSYWDHIHCTQDGWSPAVPCLRKCYFPYLENGYNQNYGRKFVQGKSIDVACHPGYALPKAQTTVTCM
ENGWSPTPRCIRVSFTL

(SEQ ID NO: 39)

An example nucleotide sequence encoding FHL1 is:

5 ATGAGACTTCTAGCAAAGATTATTTGCCTTATGTTATGGGCTATTTGTGTAGCAGAAGATTGCAATGAACTT
CCTCCAAGAAGAAATACAGAAATCTGACAGGTTCTGGTCTGACCAAACATATCCAGAAGGCACCCAGGCT
ATCTATAAATGCCGCCCTGGATATAGATCTCTTGGAATATAATAATGGTATGCAGGAAGGGAGAATGGGTT
GCTCTTAATCCATTAAGGAAATGTCAGAAAAGGCCCTGTGGACATCCTGGAGATACTCCTTTTGGTACTTTT
10 ACCCTTACAGGAGGAAATGTGTTTGAATATGGTGTAAGCTGTGTATACATGTAATGAGGGGTATCAATTG
CTAGGTGAGATTAATTACCGTGAATGTGACACAGATGGATGGACCAATGATATTCCTATATGTGAAGTTGTG
AAGTGTTTACCACTGACAGCACCAGAGAATGGAAAAATTGTCAGTAGTGCAATGGAACCAGATCGGGAATAC
CATTTTGGACAAGCAGTACGGTTTGTATGTAACCTCAGGCTACAAGATTGAAGGAGATGAAGAAATGCATTGT
TCAGACGATGGTTTTTGGAGTAAAGAGAAACCAAAGTGTGTGGAATTTTCATGCAATCCCCAGATGTTATA
AATGGATCTCTATATCTCAGAAGATTATTTATAAGGAGAATGAACGATTTCAATATAAATGTAACATGGGT
15 TATGAATACAGTGAAAGAGGAGATGCTGTATGCACTGAATCTGGATGGCGTCCGTTGCCTTCATGTGAAGAA
AAATCATGTGATAATCCTTATATTCCAAATGGTGACTACTCACCTTTAAGGATTAAACACAGAACTGGAGAT
GAAATCAGTACCACTGAGAAATGGTTTTTATCCTGCAACCCGGGGAATACAGCAAAATGCACAAGTACT
GGCTGGATACCTGCTCCGAGATGTACCTTGAACCTTGTGATTATCCAGACATTAAACATGGAGGTCTATAT
CATGAGAATATGCGTAGACCATACTTTCCAGTAGCTGTAGGAAAATATTACTCCTATTACTGTGATGAACAT
20 TTTGAGACTCCGTCAGGAAGTTACTGGGATCACATTTCATTGCACACAAGATGGATGGTCGCCAGCAGTACCA
TGCCTCAGAAAATGTTATTTTCCCTATTTGGAAAATGGATATAATCAAAATTATGGAAGAAAGTTTGTACAG
GGTAAATCTATAGACGTTGCCCTGCCATCCTGGCTACGCTCTTCCAAAAGCGCAGACCACAGTTACATGTATG
GAGAATGGCTGGTCTCCTACTCCAGATGCATCCGTGTGCTCAGCTTTACCCCTCTGA

(SEQ ID NO: 40)

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

A preferred nucleotide sequence encoding FHL1 is SEQ ID NO: 41.

ATGCGCCTCCTGGCCAAGATCATCTGCCTCATGCTGTGGGCCATCTGCGTGGCTGAGGACTGCAATGAGCTG
CCGCCCAGGAGGAACACAGAGATCCTGACAGGGAGCTGGTCTGACCAAGACCTACCCTGAGGGCAGCCAGGCG
ATCTACAAGTCCCGCCGGGCTACAGGAGCCTGGGGAACATCATCATGGTGTGTAGAAAGGGCGAATGGGTG
30 GCCCTCAACCTGAGGAAGTGCCAGAAGCGGCCCTGTGGCCACCCCGGGGACACACCCTTCGGGACCTTC
ACCCTGACCGGCGGCAATGTGTTTGAAGTACGGCGTGAAGGCTGTCTACACATGCAACGAGGGGTACCAGCTG
CTGGGCGAGATTAATACTACCGGAGTGTGACACCGATGGGTGGACCAACGACATTCCCATCTGTGAGGTGGTC
AAGTGTCTCCCGTGTGACAGCCCCAGAAAATGGCAAAATCGTGAGCAGCGCCATGGAGCCTGACCGCAATAT
CACTTTGGGCGAGCCGCTGAGGTTTGTGTGCAACTCGGGCTACAAAATTGAAGGTGATGAGGAGATGCACTGC
35 AGCGATGATGGCTTCTGGTCCAAGGAGAAGCCCAATGTGTGGAGATCTCCTGCAAGTCTCCCGACGTGATC
AACGGCAGCCCAATCAGCCAGAAGATTATTTACAAAGAGAACGAGCGCTTCCAGTACAAGTGTAAACATGGGC
TATGAGTATTGAGAGAGGGGAGATGCCGTCTGCACTGAGAGCGGCTGGAGACCACTGCCTAGCTGCGAGGAA
AAGAGTTGTGACAACCCCTTACATCCCAAATGGCGACTACTCCCCTCTGCGGATCAAACACCGGACCGGGGAT
GAAATCACCTATCAGTGCCGCAATGGATTCTACCCGGCCACCCGCGGCAACACCGCCAAATGCACCAGCACA
40 GGCTGGATCCCCGCCGCCCTGTACGCTGAAGCCTTGCGACTATCCAGACATCAAGCACGGAGGCCTGTAC
CACGAAAACATGCGGCGGCCTTATTTCCCTGTGGCAGTGGGGAAGTACTACAGCTACTACTGCGACGAGCAC
TTCGAGACCCCCTCTGGCTCCTACTGGGACCACATCCACTGCACACAGGACGGCTGGTCTCCAGCTGTGCCC
TGCCTGAGGAAATGCTACTTCCCCTACCTGGAGAACGGATACAACCAGAACTATGGCCGCAAGTTCGTGCAG
GGCAAGAGCATCGATGTGGCCTGCCACCCTGGCTACGCCCTGCCCAAGGCCAGACAACCTGTGACCTGCATG
45 GAGAATGTTGAGGCCCCACCCGCGCTGCATCCGGGTGTCTTCACGCTC

(SEQ ID NO: 41)

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: 40 or 41,

preferably SEQ ID NO: 41. Preferably, the protein encoded by the nucleotide sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 39.

In other embodiments, the nucleotide sequence encoding FHL1 is SEQ ID NO: 40 or 41, preferably SEQ ID NO: 41.

5 Complement Receptor 1 (CR1)

Complement Receptor 1 (CR1) mediates cellular binding to particles and immune complexes that have activated complement.

In some embodiments, the CR1 is human CR1.

In some embodiments, the amino acid sequence of CR1 is SEQ ID NO: 42. Preferably, a variant or fragment substantially retains a functional activity of the protein represented by SEQ ID NO: 42

MGASSPRSPPEVGPAPGLPFCCGGSLAVVLLALPVAWGQCNAPEWLPFARPTNLTDEFEFPIGTYLNYECRP
 GYSGRPFSSIICLNKNSVWTGAKDRCKRKRSCRNPPDPVNGMVHVIKGIQFGSQIKYSCTKGYRLIGSSSATCIISGD
 TVIWDNETPICDRI PCGLPPTITNGDFISTNRENFHYGSVVTYRCNPGSGGRKVFELVGEPsiYCTSNDDQVGIW
 15 SGFAPQCIIPNKCTPPNVENGILVSDNRSLSLNEVVEFRCQPGFVMKGPRRVKCQALNKWEPELPSCSRVCQPP
 PDVLHAERTQRDKDNFSPGQEVFYSCEPGYDLRGAASMRCTPQGDWSPAAPTCEVKSCDDFMGQLLNGRVLPVNL
 LQLGAKVDFVCDEGFQLKGSSASVCVLAGMESLWNSVVPVCEQIFCPSPPIVINGRHTGKPLEVFPFGKTVNYTC
 DPHDRGTSFDLIGESTIRCTSDPQGNVWSSPAPRCGILGHCQAPDHFLFAKLKTQTNASDFPIGTSLKYECRP
 EYYGRPFSSITCLDNLVWSSPKDVCKRKSKCTPPDPVNGMVHVITDIQVGSRIYNSCTTGHRLLIGHSSAECILSGNA
 20 AHWSTKPPICQRI PCGLPPTIANGDFISTNRENFHYGSVVTYRCNPGSGGRKVFELVGEPsiYCTSNDDQVGIWS
 GPAPQCIIPNKCTPPNVENGILVSDNRSLSLNEVVEFRCQPGFVMKGPRRVKCQALNKWEPELPSCSRVCQPPP
 DVLHAERTQRDKDNFSPGQEVFYSCEPGYDLRGAASMRCTPQGDWSPAAPTCEVKSCDDFMGQLLNGRVLPVNL
 LQLGAKVDFVCDEGFQLKGSSASVCVLAGMESLWNSVVPVCEQIFCPSPPIVINGRHTGKPLEVFPFGKAVNYTCD
 PHDRGTSFDLIGESTIRCTSDPQGNVWSSPAPRCGILGHCQAPDHFLFAKLKTQTNASDFPIGTSLKYECRPE
 25 EYYGRPFSSITCLDNLVWSSPKDVCKRKSKCTPPDPVNGMVHVITDIQVGSRIYNSCTTGHRLLIGHSSAECILSGNT
 AHWSTKPPICQRI PCGLPPTIANGDFISTNRENFHYGSVVTYRCNLGSRGRKVFELVGEPsiYCTSNDDQVGIWS
 GPAPQCIIPNKCTPPNVENGILVSDNRSLSLNEVVEFRCQPGFVMKGPRRVKCQALNKWEPELPSCSRVCQPPP
 EILHGEHTPSHQDNFSPGQEVFYSCEPGYDLRGAASLHCTPQGDWSPAAPRCVAVKSCDDFLGQLPHGRVLPFNL
 LQLGAKVDFVCDEGFRLKGSSVSHCVLVGMRSWNSVVPVCEHIFCPNPPAILNGRHTGTSPGDI PYGKEISYTC
 30 PHDRGTMFNLIGESTIRCTSDPHGNGVWSSPAPRCVAVKSCDDFLGQLPHGRVLPFNL
 RPYGFGKMFISISLENLVWSSVEDNCRKSCGPPPEPFNGMVHINTDTQFGSTVNYSCNEGFRLIGSPSTTCLVS
 GNNVTWDDKAPICEIISCEPPPTISNGDFYSNNRTSFHNGTVVITYQCHTGPDGEQLFELVGERSIYCTSKDDQVG
 VWSSPPPRCISTNKCTAPEVENAIRVPGNRSFFSLTEIIRFRCQPGFVMVGSHTVQCQTNGRWGPKLPHCSRVCC
 PPPEILHGEHTLSHQDNFSPGQEVFYSCEPSYDLRGAASLHCTPQGDWSPAAPRCTVKSCDDFLGQLPHGRVLLP
 35 LNLQLGAKVDFVCDEGFRLKGRSASHCVLAGMKALWNSVVPVCEQIFCPNPPAILNGRHTGTSPGDI PYGKEISY
 ACDTHPDRGTMFNLIGESSIRCTSDPQGNVWSSPAPRCVAVKSCDDFLGQLPHGRVLLP
 CDPGYLLVGKGFIFCTDQGIWSQLDHYCKEVNCSFPLFMNGISKELEMKKVYHYGDYVTLKCEDGYTLEGSPWSQ
 CQADDRWDPPPLAKCTSRTHDALIVGTLSGTIFFILLIFLSWIIILKHKRGNNAHENPKEVAIHLHSQGGSSVHPR
 TLQTNEENSRLVP

40 (SEQ ID NO: 42)

Membrane cofactor protein (MCP)

MCP acts as a cofactor for CFI.

In some embodiments, the MCP is human MCP.

In some embodiments, the amino acid sequence of MCP is SEQ ID NO: 43 or a variant or fragment thereof. Preferably, variant or fragment substantially retains a functional activity of the protein represented by SEQ ID NO: 43

5 MEPPGRRECPFPFSWRFPGLLLAAMVLLLYSFSDACEEPPTFEAMELIGKPKPYEIGERVDYKCKKGYFYIPLA
 THTICDRNHTWLPVSDDACYRETCPIYIRDPLNGQAVPANGTYEFGYQMHFICNEGYLIGEEILYCELKGSVAIW
 SGKPPICEKVLCTPPPKIKNGKHTFSEVEVFEYLDVAVTSCDPAPGDPFSLIGESTIYCGDNSVWSRAAPECKV
 VKCRFPVVENGKQISGFGKKFYKATVMFECDKGFYLDGSDTIVCDNSTWDPPVPKCLKVLPPSSTKPPALSHS
 10 VSTSSTTKSPASSASGPRPTYKPPVSNYPGYPKPEEGILDSDLVWVIAVIVIAIVGVAVICVVPYRYLQRRKKK
 GTYLTDETHREVKFTSL
 (SEQ ID NO: 43)

Linkers

In preferred embodiments, the nucleotide sequence encoding the anti-VEGF entity is upstream of the nucleotide sequence encoding the negative complement regulator. In other
 15 embodiments, the nucleotide sequence encoding the negative complement regulator is upstream of the nucleotide sequence encoding the anti-VEGF entity.

As used herein, “upstream” and “downstream” both refer to relative positions in DNA or RNA. Each strand of DNA or RNA has a 5' end and a 3' end and, by convention, “upstream” and “downstream” relate to the 5' to 3' direction respectively in which RNA transcription takes
 20 place. For example, when considering double-stranded DNA, “upstream” is toward the 5' end of the coding strand and downstream is toward the 3' end of the coding strand.

In some embodiments, the nucleotide sequences encoding the anti-VEGF entity and the negative complement regulator 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
 25 is defined by a Furin cleavage site, GSG, 11a1D and an F2A sequence.

In some embodiments, the linker is SEQ ID NO: 44.

CGAAGGAAACGAGGAAGCGGAGAGCCAGACACAAACAGAAAATTGTGGCACCGGTGAAACAGACTTTGAAT
 TTTGACCTTCTCAAGTTGGCGGAGACGTCGAGTCCAACCTGGGCC
 (SEQ ID NO: 44)

30 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: 44. Preferably, the linker substantially retains a functional activity of SEQ ID NO: 44.

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) an anti-VEGF entity; and (ii) a negative complement regulator, or nucleotide sequences encoding therefor, in admixture. Alternatively, the product may, for example, be a kit comprising preparations of (i) an anti-VEGF entity; and (ii) a negative complement regulator, or nucleotide sequences encoding therefor, and, optionally, instructions for the simultaneous, sequential or separate administration of the 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; 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: 805-7). 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 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.

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.

Polynucleotides may be delivered to cells by plasmid transfection or electroporation; for example as non-viral vectors such as naked DNA plasmids, bacterial artificial chromosomes, mini-circle technologies. These entities can also package larger transgenes than AAV vectors but may at a slight disadvantage of being less stable, bioavailability and target specificity. For example, Eyeevensys uses a naked DNA electroporation platform for delivering therapeutic transgenes to muscles/eye etc.

In some embodiments, the polynucleotide comprises or consists of the nucleotide sequence of SEQ ID NO: 45, 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.

CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCAGTGAGCGAG
CGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTTCTGGCGCGCCGGAGTTCGCGTTACATAACT
TACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCAT
AGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACA
TCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCA
GTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCG
GTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTTGACGT
CAATGGGAGTTTGTGTTTGGCACCAAAATCAACGGGACTTTCAAAATGTCGTAACAACCTCCGCCCCATTGACGCA
AATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAG
ACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGACTAGTGCCACCATGAACTGCTGCATGTCTTCCTC

CTCTTCCTGTGCTTCCACCTCCGTTTTCTGTAAAGTCACCTACACTAGCCAGGAGGATCTGGTGGAGAAGAAATGC
 CTGGCCAAGAAGTATACCCACCTGAGCTGCGACAAAGTGTCTGCCAGCCCTGGCAACGCTGCATTGAAGGTACT
 TGTGTGTGCAAGCTGCCCTACCACTGCCCCAAGAACGGCAGCGCCGTGTGTGCCACCAACAGGAGGAGCTTCCCC
 5 ACCTACTGCCAGCAGAAGAGCCTGGAATGCCTCCACCTGGCACCAGTTTCTGAACAACGGGACCTGCACAGCC
 GAGGGGAAATTCAGCGTCTCCCTCAAGCACGGCAATACAGACTCCGAGGGCATTTGTGGAAGTGAAGCTGGTGGAC
 CAGGACAAGACCATGTTTCATCTGCAAAAGCAGCTGGTCCATGCGGGAGGCCAATGTCGCCTGCCTGGACCTGGGC
 TTCCAGCAGGGCGCTGATACACAGCGCGCTTTAAACTCAGTGACCTCAGCATCAACAGCACTGAGTGTCTGCAC
 GTGCACTGCCGGGGCCTGGAGACCAGCCTGGCTGAGTGCACCTTCACCAAGCGCAGGACCATGGGCTACCAGGAT
 10 TTTGTCAGATGTGGTCTGCTACACCCAGAAGGCAGACAGCCCCATGGATGACTTCTTCCAGTGTGTCAATGGCAAG
 TACATTTCCCAGATGAAGGCTTGTGACGGGATCAATGATTGCGGGGATCAGAGCGATGAGCTCTGCTGCAAGGCC
 TGCCAAGGGAAGGGCTTTTACTGTAAGTCTGGGGTGTGCATCCCTTCTCAGTATCAGTGCAACGGGAGAGGTGGAC
 TGATCACTGGGGAGGACGAGGTGGGCTGTGCTGGCTTCGCCTCTGTGGCCAGGAGGAGACAGAGATCCTCACA
 GCTGACATGGATGCAGAGCGGCGCGCATCAAGAGTCTGCTCCCAAAGCTCTCCTGCGGCGTTAAGAATCGCATG
 15 CACATCCGGAGGAAGCGGATCGTTGGAGGCAAACGGGCTCAGCTGGGGGACTTGCCGTGGCAGGTGGCCATCAAA
 GATGCCTCCGGAATCACCTGTGGTGGCATCTACATCGGCGGCTGCTGGATCCTGACCGCGCCGCACTGCCTTCGG
 GCCAGCAAGACTCACCGCTACCAGATCTGGACCACCGTGGTGGATTGGATTACCCCGACCTGAAGAGGATTGTC
 ATTGAGTATGTGACCGCATCATCTTCCATGAAACTACAATGCCGGGACGTATCAGAACGACATCGCCCTCATC
 GAGATGAAGAAGGATGGGAACAAGAAGGACTGTGAGCTGCCTCGCTCCATCCCCGCTGTGTACCATGGTCTCCG
 20 TACCTGTTCCAGCCAAATGACACATGCATCGTGAGCGGCTGGGGCCGCGAGAAAGACAACGAGAGGGTCTTCTCC
 CTGCACTGGGGTGAAGTCAAGCTGATCAGCAACTGCTCCAAGTTCTACGGCAACCGCTTCTATGAGAAGGAGATG
 GAGTGCGCCGCGACCTATGACGGCAGCATTGACGCGTGCAAGGGAGACAGTGGGGGCCCCCTGCTGTGCATGGAC
 GCCAACAATGTGACCTACGTGTGGGGAGTTGTGTCTGGGGCGAGAACTGTGGCAAGCCTGAGTTCGCCGGCGTG
 TACACAAAGGTGGCAAATATTTTACTGGATCTCCTATCACGTTGGCAGGCCCTTCATTTACAGTACAACGTA
 25 CGAAGGAAACGAGGAAGCGGAGAGCCAGACACAAACAGAAAATTTGTGGCACCCTGAAACAGACTTTGAATTTT
 GACCTTCTCAAGTTGGCGGGAGACGTGAGTCCAACCTGGGCCCATGCGCCTGCTGGCCAAGATCATCTGCCTG
 ATGCTGTGGGCCATCTGCGTGGCCAGCGACACCGGCGGCCCTTCTGTGGAGATGTACAGCGAGATCCCCGAGATC
 ATCCACATGACCGAGGGCCGCGAGCTGGTGTATCCCCGCGTGACCGAGCCCCAACATCACCGTGACCTGAAG
 AAGTTCCCCCTGGACACCTGATCCCCGACGGCAAGCGCATCATCTGGGACAGCCGCAAGGGCTTCATCATCAGC
 30 AACGCCACCTACAAGGAGATCGGCCTGCTGACCTGCGAGGCCACCGTGAACGGCCACCTGTACAAGACCAACTAC
 CTGACCCACCCGAGACCAACACCATCATCGACGTGGTGTGCTGAGCCCCAGCCAGCGCATCGAGCTGAGCGTGGGC
 GAGAAGCTGGTGTGAATGCACCGCCCGCACCGAGCTGAACGTGGGCATCGACTTCAACTGGGAGTACCCAGC
 AGCAAGCACCAGCACAAGAAGCTGGTGAACCGCGACCTGAAGACCCAGAGCGGCGAGATGAAGAAGTTCCTG
 AGCACCTGACCATCGACGCGGTGACCCGACGCGACAGGGCCTGTACACCTGCGCCGCGCAGCAGCGGCCTGATG
 35 ACCAAGAAGAACAGACACCTTCGTGCGCGTGACGAGAAGGACAAGACCCACACCTGCCCCCTGCCCCGCCCC
 GAGCTGTGGCGGCCCGCCAGCGTGTTCCTGTTCCCCCAAGGCCAAGGACACCTGATGATCAGCCGACCCCC
 GAGGTGACCTGCGTGGTGGACGTGAGCCACGAGACCCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTG
 GAGGTGCACAACGCCAAGACCAAGCCCCGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGGAGCGTGTGACC
 40 GTGCTGCACCAAGGACTGGTGAACGGCAAGGAGTACAAGTGAAGGTGAGCAACAAGGCCCTGCCCGCCCCCATC
 GAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACCTGCCCCCAGCCGCGACGAG
 CTGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAG
 AGCAACGGCCAGCCCGAGAACAACTACAAGACACCCCCCGTGTGGACAGCGACGGCAGCTTCTTCTGTATC
 AGCAAGCTGACCGTGACCAAGAGCCGCTGGCAGCAGGGCAACGTGTTTCACTGACGCGTGTGACGAGGCCCTG
 45 CACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCCGCTAACTCGAGAATCAACCTCTGGATTACAAAATT
 TGTGAAAGATTGACTGGTATTTCTTAATATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTAAATGCCTTTG
 TATCATGCTATTGCTTCCCGTATGGCTTTTCAATTTCTCCTCCTTGTATAAATCCTGGTTAGTTCTTGCCACGGCG
 GAACTCATCGCCGCTGCCCTGCCCCGTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTGC
 50 CTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCGTGCTTCTTACCTGAAAGGTGCCACTCCCACTG
 TCCTTTCTAATAAAATGAGGAAATTCATCGCATGTCTGAGTAGGTGTCTTCTATTCTGGGGGTGGGGTGG
 GGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGGCGGCC
 GCAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTGCGCGCTGCTCGCTCACTGAGGCCGGGCGACCAAA
 GTGCGCCGACGCGCGGGCTTTGCCGGGCGGCCTCAGTGAGCGAGCG

(SEQ ID NO: 45)

In some embodiments, the polynucleotide comprises or consists of the nucleotide sequence
 of SEQ ID NO: 46, or a nucleotide sequence that has at least 75%, 80%, 85%, 86%, 87%,
 55 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity
 thereto.

CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCAGTGAGC
GAGCGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTTCTTGGCGCGCCGGAGTTCCGCGTTAC
ATAACTTACGGTAAATGGCCCCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTA
5 TGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCA
CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCG
CTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGC
TATTACCATTGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTC
AAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTGGCACCAAAATCAACGGGACTTTCCAAAATGTCTG
10 TAACAACCTCCGCCCATTGACGCAATGGGCGGTAGGCGGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCG
TTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGACTAGT
ACCGCCCGCACCAGCTGAACGTGGGCATCGACTTCAACTGGGAGTACCCAGCAGCAAGCACCAGCAACAG
CGCCCCCTTCGTGGAGATGTACAGCGAGATCCCCGAGATCATCCACATGACCGAGGGCCGCGAGCTGGTGATC
CCCTGCCGCGTGACCGACCCCAACATCACCGTGACCCTGAAGAAGTTCCCCCTGGACACCCTGATCCCCGAC
15 GGCAAGCGCATCATCTGGGACAGCCGCAAGGGCTTCATCATCAGCAACGCCACCTACAAGGAGATCGGCGCTG
CTGACCTGCGAGGCCACCGTGAACGGCCACCTGTACAAGACCAACTACCTGACCCACCGCCAGACCAACACC
ATCATCGACGTGGTGCTGAGCCCCAGCCACGGCATCGAGCTGAGCGTGGGCGAGAAGCTGGTGCTGAACCTGC
ACCGCCCGCACCAGCTGAACGTGGGCATCGACTTCAACTGGGAGTACCCAGCAGCAAGCACCAGCAACAG
AAGCTGGTGAACCGCGACCTGAAGACCCAGAGCGGCGAGAGATGAAGAAGTTCTTGAGCACCCCTGACCATC
20 GACGGCGTGACCCGAGCGACCGAGGGCTGTACACCTGCGCCGCCAGCAGCGGCGCTGATGACCAAGAAGAAC
AGCACCTTCGTGCGCTGTCACGAGAAGGACAAGACCCACACCTGCCCCCTGCCCCGCCCGGAGCTGCTG
GGCGGCCCCAGCGTGTCTGTTCCCCCCCCAAGCCCAAGGACACCCCTGATGATCAGCCGACCCCCGAGGTG
ACCTGCGTGGTGGTGGAGCTGAGCCACGAGGACCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAG
GTGCACACGCCCAAGACCAAGCCCCGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGAGCGTGCTGACC
25 GTGCTGCACAGGACTGGCTGAACGGCAAGGAGTACAAGTGAAGGTGAGCAACAAGGCCCTGCCGCCCCCC
ATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACCTGCCCCCCAGCCGC
GACGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTTACCCAGCGACATCGCCGTG
GAGTGGGAGAGCAACGGCCAGCCCGAGAACAATAACAAGACACCCCCCGTGTGGACAGCGACGGCAGC
TTCTTCTGTACAGCAAGCTGACCGTGGACAAGAGCCGCTGGCAGCAGGGCAACGTGTTACGTGACGCGTG
30 ATGCACAGGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCCGGCTAACGAAGGAACGA
GGAAGCGGAGAAGCCAGACACAACAGAAAATTGTGGCACCGGTGAAACAGACTTTGAATTTTGACCTTCTC
AAGTTGGCGGGAGACGTGAGTCCAACCCCTGGGCCCATGAAACTGCTGCATGTCTTCTCTCTTCTCTGTGC
TTCCACCTCCGTTTTCTGTAAAGTCACTACCTAGCCAGGAGGATCTGGTGGAAGAAGAAATGCCTGGCCAA
AAGTATACCCACCTGAGCTGCGACAAAGTGTCTGCCAGCCCTGGCAACGCTGCATTGAAGGTACTTGTGTG
35 TGCAAGCTGCCCTACAGTGCCCCAAGACGGCAGGCGCTGTGTGCCACCAACAGGAGGACTTCCCCACC
TACTGCCAGCAGAAGAGCCTGGAATGCCTCCACCTGGCACCAAGTTTCTGAACAACGGGACCTGCACAGCC
GAGGGGAAATTACGCGTCTCCCTCAAGCACGGCAATACAGACTCCGAGGGCATTGTGGAAGTGAAGCTGGTG
GACCAGGACAAGACCATGTTTCATCTGCAAAAGCAGCTGGTCCATGCGGGAGGCCAATGTGCGCTGCCTGGAC
40 CTGGGCTTCCAGCAGGGCGCTGATACACAGCGCGCTTTAAACTCAGTGACCTCAGCATCAACAGCACTGAG
TGTCTGCACGTGCACTGCCGGGGCTGGAGACCAGCCTGGCTGAGTGCACCTTCAACAGCGCAGGACCATG
GGCTACAGGATTTTGAGATGTGGTCTGTACACCCAGAAGGCAGACAGCCCCATGGATGACTTCTTCCAG
TGTGTCAATGGCAAGTACATTTCCAGATGAAGCTTGTGACGGGATCAATGATTGCGGGGATCAGAGCGAT
GAGCTCTGCTGCAAGGCTGCCAAGGGAAGGGCTTTCACTGTAAGTCTGGGGTGTGCATCCCTTCTCAGTAT
CAGTGCAACGGAGAGGTGGACTGCATCACTGGGAGGACGAGGTGGGCTGTGCTGGCTTCGCTCTGTGGCC
45 CAGGAGGAGACAGAGATCCTCACAGCTGACATGGATGCAGAGCGGCGGCGCATCAAGAGTCTGCTCCCAAAG
CTCTCTGCGCGTTAAGAATCGCATGCACATCCGGAGGAAGCGGATCGTTGGAGGCAACGGGCTCAGCTG
GGGACTTGGCGTGGCAGGTGGCCATCAAAGATGCCTCCGGAATCACCTGTGGTGGCATCTACATCGCGCGC
TGCTGGATCCTGACCGCGCCCACTGCCTTCGGGCCAGCAAGACTCACCGCTACCAGATCTGGACCACCGTG
GTGGATTGGATTACCCCGACCTGAAGAGGATTGTCAATTGAGTATGTGACCGCATCATCTTCCATGAAAAC
50 TACAATGCCGGGACGTATCAGAACGACATCGCCCTCATCGAGATGAAGAAGGATGGGAACAAGAAGGACTGT
GAGCTGCCTCGCTCCATCCCCGCCTGTGTACCATGGTCTCCGTACCTGTTCCAGCCAAATGACACATGCATC
GTGAGCGGCTGGGGCCGCGAGAAAGACAACGAGAGGGTCTTCTCCCTGCAGTGGGGTGAAGTCAAGCTGATC
AGCAACTGCTCCAAGTTCTACGGCAACCGCTTCTATGAGAAGGAGATGGAGTGCGCCGGCACCTATGACGGC
AGCATTGACGCGTGCAAGGGAGACAGTGGGGGCCCCCTGGTCTGCATGGACGCCAACAATGTGACCTACGTG
55 TGGGGAGTTGTGTCTTGGGGCGAGAACTGTGGCAAGCCTGAGTTCGCGGGCGTGACACAAAGGTGGCAAC
TATTTTGAAGTGGATCTCCTATCACGTTGGCAGGCGCTTCATTTACAGTACAACGTACTCGAGAATCAACCT
CTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACATATGTTGCTCCTTTTACGCTATGTGGATAC
GCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCGATATGGCTTTTCAATTTCTCCTCTGTATAAATCC
TGGTTAGTTCTTGGCAGGCGGAACCTCGCCGCTGCTTGGCAGGCTGCTGGACAGGCTCGGCTGTG
60 GGCATGACAATTCCGTGGTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCCT
TGACCTGGAAGGTGCCACTCCCACTGTCTTCTAATAAAATGAGGAAATGTCATCGCATTGTCTGAGTA

GGTGTCTATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGC
ATGCTGGGGATGCGGTGGGCTCTATGGGCGGCCGAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCT
GCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCACGCCCGGGCTTTGCCCGGGCGGCCTC
AGTGAGCGAGCG

5 (SEQ ID NO: 46)

In some embodiments, the polynucleotide comprises or consists of the nucleotide sequence of SEQ ID NO: 47, 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.

10 CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCAGTGAGCGAG
CGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCTTGGCGCGCCGAGTTCGCGGTTACATAACT
TACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCAT
15 AGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACA
TCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCA
GTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATCGG
GTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTGACGT
CAATGGGAGTTTGTGGTGGCACCAAATCAACGGGACTTTCCAAATGTCGTAACTCCGCCCCATTGACGCA
AATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAG
20 ACGCCATCCACGTGTTTTGACCTCCATAGAAGACACCGACTAGTGCCACCATGAAACTGCTGCATGTCTTCCTC
CTCTTCTGTGCTTCCACCTCCGTTTCTGTAAAGTCACCTACACTAGCCAGGAGGATCTGGTGGAGAAGAAATGC
CTGGCCAAGAAGTATACCCACCTGAGCTGCGACAAAGTGTCTGCGCAGCCCTGGCAACGCTGCATTGAAGGTACT
TGTGTGTGCAAGCTGCCCTACCAAGTACGCCCCAAGAACGGCACGGCCGTGTGTGCCACCAACAGGAGGAGCTTCCCC
ACCTACTGCCAGCAGAAGAGCCTGGAATGCCTCCACCTGGCACCAAGTTTCTGAACAACGGGACCTGCACAGCC
25 GAGGGGAAATTCAGCGTCTCCCTCAAGCACGGCAATACAGACTCCGAGGGCATTGTGGAAGTGAAGCTGGTGGAC
CAGGACAAGACCATGTTCTGCAAAAGCAGCTGGTCCATGCGGGAGGGCAATGTCGCCTGCCTGGACCTGGGC
TTCCAGCAGGGCGCTGATACACAGCGCCGCTTTAAACTCAGTGACCTCAGCATCAACAGCACTGAGTGTCTGCAC
GTGCACTGCCGGGGCCTGGAGACCAGCCTGGCTGAGTGCACCTTCACCAAGCGCAGGACCATGGGCTACCAAGGAT
TTTGAGATGTGGTCTGCTACACCCAGAAGGCAGACAGCCCCATGGATGACTTCTTCCAGTGTGTCAATGGCAAG
30 TACATTTCCAGATGAAGGCTTGTGACGGGATCAATGATTGCGGGGATCAGAGCGATGAGCTCTGCTGCAAGGCC
TGCCAAGGGAAGGGCTTTCACTGTAAGTCTGGGGTGTGCATCCCTTCTCAGTATCAGTGCAACGGAGAGGTGGAC
TGCATCACTGGGGAGGACGAGGTGGGCTGTGCTGGCTTCGCCTCTGTGGCCAGGAGGAGACAGAGATCCTCACA
CTGACATCGGATGACAGCGCGCGCATCAAGACTGCTGCTCCCAAGCTCTCCTGCGGCGTTAAGAATCGCATG
CACATCCGGAGGAAGCGGATCGTTGGAGGCAACGGGCTCAGCTGGGGGACTTGCCGTGGCAGGTGGCCATCAAA
35 GATGCCTCCGAATCACCTGTGGTGGCATCTACATCGGCGGCTGTGGATCCTGACCGCGCCCACTGCCTTCGG
GCCAGCAAGACTCACCGCTACCAGATCTGGACCACCGTGGTGGATTGGATTACCCCGACCTGAAGAGGATTGTC
ATTGAGTATGTCGACCGCATCATCTTCCATGAAACTACAATGCCGGGACGTATCAGAACGACATCGCCCTCATC
GAGATGAAGAAGGATGGGAACAAGAAGGACTGTGAGCTGCCTCGCTCCATCCCCGCTGTGTACCATGGTCTCCG
TACCTGTTCCAGCCAAATGACACATGCATCGTGAGCGGCTGGGGCCGCGAGAAAGACAACGAGAGGGTCTTCTCC
40 CTGCACTGGGGTGAAGTCAAGCTGATCAGCAACTGCTCCAAGTCTACGGCAACCGCTTCTATGAGAAGGAGATG
GAGTGCGCCGGCACCTATGACGGCAGCATTGACGCGTGCAAGGGAGACAGTGGGGGCCCCCTGGTCTGCATGGAC
GCCAACAATGTGACCTACGTGTGGGAGTTGTGTCTGGGGCGAGAACTGTGGCAAGCCTGAGTTCGCGGGCGTG
TACACAAAGGTGGCAAATATTTTACTGGATCTCCTATCACGTTGGCAGGCCCTTCATTTACAGTACAACGTA
CGAAGGAACGAGGAAGCGGAGAGCCAGACACAACAGAAAATTGTGGCACCAGTGAACAGACTTTGAATTTT
45 GACCTTCTCAAGTTGGCGGGAGACGTGAGTCCAACCTGGGCCCATGCGCCTGCTGGCCAAAGTATCATCTGCTG
ATGCTGTGGGCCATCTGCGTGGCCAGCGACACCGGCCGCCCTTCGTGGAGATGTACAGCGAGATCCCCGAGATC
ATCCACATGACCGAGGGCCGCGAGCTGGTGATCCCCGCGCGTGACCGCCCCAACATCACCGTGACCTGAAG
AAGTTCCCCCTGGACACCTGATCCCCGACGGCAAGCGCATCATCTGGGACAGCCGCAAGGGCTTCATCATCAGC
AACGCCACCTACAAGGAGATCGGCCTGCTGACCTGCGAGGCCACCGTGAACGGCCACCTGTACAAGACCAACTAC
50 CTGACCCACCGCCAGACCAACACCATCATCGACGTGGTGTGAGCCCCAGCCACGGCATCGAGCTGAGCGTGGGC
GAGAAGCTGGTGTGCTGAACGTGACCGCCCGCAGCAGAGTGAACGTGGGCATCGACTTCAACTGGGAGTACCCAGC
AGCAAGCACCAGCACAAGAAGCTGGTGAACCGCGACCTGAAGACCCAGAGCGGCAGCGAGATGAAGAAGTTCCTG
AGCACCTGACCATCGACGGCGTGACCCGCGAGCGACAGGGCCTGTACACCTGCGCCGCCAGCAGCGGCCTGATG
ACCAAGAAGAACAGCACCTTCGTGCGCGTGACAGAGAGGACAAGACCCACACCTGCCCCCTGCCCCGCCCCC
55 GAGCTGCTGGGCGGCCCCAGCGTGTCTCTGTTCCCCCAAGCCCAAGGACACCCCTGATGATCAGCCGCACCCCC

GAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTG
GAGGTGCACAACGCCAAGACCAAGCCCCGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGGAGCGTGTGACC
GTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAAGGCCCTGCCCGCCCCCATC
5 GAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACCTGCCCCCAGCCGCGACGAG
CTGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAG
AGCAACGGCCAGCCCCGAGAACAACCTACAAGACACCCCCCGTGGTGGACAGCGACGGCAGCTTCTTCTGTAC
AGCAAGCTGACCGTGGACAGAGCCGCTGGCAGCAGGGCAACGTGTTGAGCTGCAGCGTGATGCACGAGGCCCTG
CACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCCGGCTAACTCGAGGTGCCTTCTAGTTGCCAGCCATCT
10 GTTGTGTTGCCCTCCCCCTGCCTTCTTACCCCTGGAAGGTGCCACTCCCCTGTCCTTTCTAATAAAATGAG
GAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGAG
GATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGGCGGCCGAGGAACCCCTAGTGATGG
AGTTGGCCACTCCCTCTCTGCGCGCTCGTCTCACTGAGGCCGGGCGACCAAAGGTGCCCCGACGCCCCGGCT
TTGCCCGGGCGGCCTCAGTGAGCGAGCG

(SEQ ID NO: 47)

15 In some embodiments, the polynucleotide comprises or consists of the nucleotide sequence
of SEQ ID NO: 48, 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.

CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCAGTGAGC
20 GAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCTGGCGCGCCGGAGTTCCGCGTTAC
ATAACTTACGGTAAATGGCCCCCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTA
TGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCA
CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCG
25 CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGC
TATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTC
AAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCTG
TAACAACTCCGCCCCATTGACGCAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCG
TTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTGGACCTCCATAGAAGACACCGACTAGT
30 GCCACCATGCGCCTGCTGGCCAAGATCATCTGCCTGATGCTGTGGGCCATCTGCGTGGCCAGCGACACCGGC
CGCCCCCTCGTGGAGATGTACAGCGAGATCCCCGAGATCATCCACATGACCGAGGGCCGCGAGCTGGTGATC
CCCTGCCGCGTGACCAAGCCCAACATCACCGTGACCTGAAGAAGTTCCCCCTGGACACCTGATCCCCGAC
GGCAAGCGCATCATCTGGGACAGCCGCAAGGGCTTCATCATCAGCAACGCCACCTACAAGGAGATCGGCCTG
TGACCTGCGTAGGGCCACCGTGAACGGCCACCTGTACAAGACCACTACCTGACCCACCGGTAGCAACACC
35 ATCATCGAGCTGGTGTGAGCCCCAGCCACGGCATCGAGCTGAGCGTGGGCGAGAAGCTGGTGTGAATGTC
ACCGCCCCGACCGAGCTGAACGTGGGCATCGACTTCAACTGGGAGTACCCAGCAGCAAGCACCAGCACAAG
AAGCTGTTGAACCGCGACCTGAAGACCCAGAGCGCGAGAGATGAAGAAGTTCTGAGCACCTGACCATC
GACGGCGTGACCCGCGAGCGACAGGGCCTGTACACCTGCGCCGCCAGCAGCGGCGCTGATGACCAAGAAGAAC
AGCACCTTCGTGCGCGTGCACGAGAAGGACAAGACCCACACCTGCCCCCTGCCCCGCCCGAGCTGCTG
40 GCGGCCCGCAGCGTGTCTGTTCCTGTTCCCCCAAGCCCAAGGACACCTGATGATCAGCCGACCCCCGAGGTG
ACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAG
GTGCACAACGCCAAGACCAAGCCCCGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGGAGCGTGTGACC
GTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAAGGCCCTGCCCGCCCC
ATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACCTGCCCCCAGCCGC
45 GACGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCAGCGACATCGCCGTG
GAGTGGGAGAGCAACGGCCAGCCCCGAGAACAACCTACAAGACACCCCCCGTGTGGCAGCGACGGCAGC
TTCTTCTGTACAGCAAGCTGACCGTGGACAAGAGCCGCTGGCAGCAGGGCAACGTGTTGAGCTGCAGCGTG
ATGCACGAGGGCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTGAGCCCCGGCTAACGAAGGAACGA
50 GGAAGCGGAGAAGCCAGACACAAACAGAAAATTGTGGCACCGGTGAAACAGACTTTGAATTTTGACCTTCTC
AAGTTGGCGGGAGACGTGAGTCCAACCTGGGCCCCATGAACTGCTGCATGTCTTCTCTCTCTCTGTGC
TTCCACCTCCGTTTCTGTAAAGTCACCTACCTAGCCAGGAGATCTGGTGGAGAAGAAATGCCTGGCCAAG
AAGTATACCCACCTGAGCTGCGACAAAGTGTCTGCCAGCCCTGGCAACGCTGCATTGAAGGTACTTGTGTG
TGCAAGCTGCCCTACCAGTGCCCCAAGAACGGCACGGCCGTGTGTGCCACCAACAGGAGGAGCTTCCCCACC
TACTGCCAGCAGAAGAGCCTGGAATGCCTCCACCTGGCACCAAGTTTCTGAACAACGGGACCTGCACAGCC
55 GAGGGGAATTCAGCGTCTCCCTCAAGCACGGCAATACAGACTCCGAGGGCATTGTGGAAGTGAAGCTGGTG
GACCAGGACAAGACCATGTTTCATCTGCAAAAGCAGCTGGTCCATGCGGGAGGCCAATGTCGCTGCCTGGAC

CTGGGCTTCCAGCAGGGCGCTGATACACAGCGCCGCTTTAAACTCAGTGACCTCAGCATCAACAGCACTGAG
TGTCTGCACGTGCACTGCCGGGCTGGAGACCAGCCTGGCTGAGTGCACCTTCACCAAGCGCAGGACCATG
GGCTACCAGGATTTTGCAGATGTGGTCTGCTACACCCAGAAGGCAGACAGCCCATGGATGACTTCTTCAG
TGTGTCAATGGCAAGTACATTTCCAGATGAAGCTTGTGACGGGATCAATGATTGCGGGGATCAGAGCGAT
5 GAGCTCTGCTGCAAGGCCTGCCAAGGGAAGGGCTTTCAGTGTAACTCTGGGGTGTGCATCCCTTCTCAGTAT
CAGTGCAACGGAGAGGTGGACTGCATCACTGGGAGGACGAGGTGGGCTGTGCTGGCTTCGCCTCTGTGGCC
CAGGAGGAGACAGAGATCCTCACAGCTGACATGGATGCAGAGCGGCGGCATCAAGAGTCTGCTCCCAAAG
CTCTCTGCGGCGTTAAGAATCGCATGCACATCCGGAGGAAGCGGATCGTTGGAGGCAACGGGCTCAGCTG
GGGGACTTGCCGTGGCAGGTGGCCATCAAAGATGCCTCCGGAATCACCTGTGGTGGCATCTACATCGGCGGC
10 TGCTGGATCCTGACCGCGCCCACTGCCTTCGGGCCAGCAAGACTCACCGTACCAGATCTGGACCACCGTG
GTGGATTGGATTACCCCGACCTGAAGAGGATTGTCAATTGAGTATGTCGACCGCATCATCTTCCATGAAAAAC
TACAATGCCGGGACGTATCAGAACGACATCGCCCTCATCGAGATGAAGAAGGATGGGAACAAGAAGGACTGT
GAGCTGCCTCGCTCCATCCCGCCTGTGTACCATGGTCTCCGTACCTGTTCCAGCCAAATGACACATGCATC
15 GTGAGCGGCTGGGGCGCGAGAAAGACAACGAGAGGGTCTTCTCCCTGCAGTGGGGTGAAGTCAAGCTGATC
AGCAACTGCTCCAAGTCTACGGCAACCGCTTCTATGAGAAGGAGATGGAGTGCGCCGGCACCTATGACGGC
AGCATTGACCGGTGCAAGGGAGACAGTGGGGGCCCCCTGGTCTGCATGGACGCCAACAATGTGACCTACGTG
TGGGGAGTTGTGTCTGGGGCGAGAACTGTGGCAAGCCTGAGTTCGGGGCGTGTACACAAAGGTGGCAAAAC
TATTTTGA CTGGATCCTATCACGTTGGCAGGCCCTTCATTTACAGTACAACGTACTCGAGGTGCCTTCT
AGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCTTGACCCTGGAAGGTGCCACTCCCCTGTC
20 CTTTCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCAATTCTATTCTGGGGGTGGGGT
GGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGGC
GGCCGACAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGC
GACCAAGGTGCGCCGACGCGCCGGCTTTGCCCGGGCGGCCCTCAGTGAGCGAGCG (SEQ ID NO: 48)

In some embodiments, the polynucleotide comprises or consists of the nucleotide sequence
25 of SEQ ID NO: 49, 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.

CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTGGGGCGACCTTTGGTGGCCCGGCTCAGTGAGCGAG
CGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCTTGGCGCGCCGAGTTCGGCTTACATAACT
30 TACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTGACGTCAATAATGACGTATGTTCCCAT
AGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCACTTGGCAGTACA
TCAAGTGTATCATATGCCAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCCA
GTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCG
GTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGA CTACGGGGATTTCAGTCTCCACCCCATTGACGT
35 CAATGGGAGTTTGTGTTTGGCACAAATCAACGGGACTTTCCAAAATGTGTAACAACTCCGCCCATTGACGCA
AATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAG
ACGCCATCCAGCTGTTTTGACCTCCATAGAAGACACCGACTAGTGCCACCATGCGCCTCCTGGCCAAGATCATC
TGCCCTCATGCTGTGGGCCATCTGCGTGGCTGAGGACTGCAATGAGCTGCCGCCAGGAGGAACACAGAGATCGTG
ACAGGGAGCTGGTCTGACCAGACCTACCTGAGGGCACCCAGGCGATCTACAAGTGCCGGCCGGGCTACAGGAGC
40 CTGGGGAACATCATCATGTTGTGTAGAAAGGGCGAATGGGTGGCCCTCAACCCCTGAGGAAGTGCCAGAAGCGG
CCCTGTGGCCACCCCGGGGACACACCTTTCGGGACCTTCACCCTGACCGGCGGCAATGTGTTTGA GTACGGCGTG
AAGGCTGTCTACACATGCAACGAGGGGTACCAGCTGCTGGGCGAGATTA ACTACCGGGAGTGTGACACCGATGGG
TGGACCAACGACATTCCCATCTGTGAGGTGGTCAAGTGTCTCCCGTGACAGCCCAGAAAATGGCAAAATCGTG
AGCAGCGCCATGGAGCCTGACCGCGAATATCACTTTGGGCAGGCCGTGAGGTTTGTGTGCAACTCGGGCTACAAA
45 ATTGAAGGTGATGAGGAGATGCACTGCAGCGATGATGGCTTCTGGTCCAAGGAGAAGCCCAAGATGTGTGAGATC
TCTTGAAGTCTCCCGACGTGATCAACGGCAGCCCATCGCTCAGCAGAAGATTATTTACAAAGAGACCGCTTC
CAGTACAAGTGTAAATGGGCTATGAGTATTAGAGAGGGGAGATGCCGTCTGCACTGAGAGCGGCTGGAGACCA
CTGCCTAGCTGCGAGGAAAAGAGTTGTGACAACCTTACATCCCAAATGGCGACTACTCCCTCTGCGGATCAAAA
CACCGGACCGGGGATGAAATCACCTATCAGTGCCGCAATGGATTCTACCGGCCACCCGCGGCAACACCGCCAAA
50 TGCACCAGCACAGGCTGGATCCCGCCCCCGCTGTACGCTGAAGCCTTGCGACTATCCAGACATCAAGCACGGA
GGCCTGTACCAGAAAACATGCGGCGGCCCTTATTTCCCTGTGGCAGTGGGGAAGTACTACAGTACTACTGCGAC
GAGCACTTCGAGACCCCTCTGGCTCCTACTGGGACACATCCACTGCACACAGGACGGCTGGTCTCCAGCTGTG
CCCTGCCTGAGGAAATGCTACTTCCCCTACCTGGAGAACGGATACAACCAGAACTATGGCCGCAAGTTCTGTGAG
GGCAAGAGCATCGATGTGGCTGCCACCTGGCTACGCCCTGCCAAGGCCAGACA ACTGTGACCTGCATGGAG
55 AATGGTTGGAGCCCCACCCGCGCTGCATCCGGGTGTCTTACGCTCCGAAGGAACGAGGAAGCGGAGAACCC
AGACACAAACAGAAAATTTGTGGCACCGGTGAAACAGACTTTGAATTTTGA CTCTCAAGTTGGCGGGAGACGTC

GAGTCCAACCCTGGGCCCATGCGCCTGCTGGCCAAGATCATCTGCCTGATGCTGTGGGCCATCTGCGTGGCCAGC
 GACACCGGCCGCCCTTCGTGGAGATGTACAGCGAGATCCCCGAGATCATCCACATGACCGAGGGCCGCGAGCTG
 GTGATCCCCCTGCCGCGTGACCGAGCCCCAACATCACCGTGACCCCTGAAGAAGTCCCCCTGGACACCCTGATCCCC
 GACGGCAAGCGCATCATCTGGGACAGCCGCAAGGGCTTCATCATCAGCAACGCCACCTACAAGGAGATCGGCCCTG
 5 CTGACCTGCGAGGGCCACCGTGAACGGCCACCTGTACAAGACCAACTACCTGACCCACCGCCAGACCAACACCATC
 ATCGACGTGGTGTGAGCCCCAGCCAGCGCATCGAGCTGAGCGTGGGCGAGAAGCTGGTGTGAAGTGCACCGCC
 CGCACCGAGCTGAACGTGGGCATCGACTTCAACTGGGAGTACCCAGCAGCAAGCACCAGCACAGAAGCTGGTG
 AACC CGCAGCTGAAGACCCAGAGCGGCAGCGAGATGAAGAAGTTCCTGAGCACCCCTGACCATCGACGGCGTGACC
 CGCAGCGACCAAGGGCCTGTACACCTGCGCCGCCAGCAGCGGCCCTGATGACCAAGAAGAACAGCACCTTCGTGCGC
 10 GTGCACGAGAAGGACAAGACCCACACCTGCCCCCCTGCCCCGCCCCGAGCTGCTGGGCGGCCCCAGCGTGTTT
 CTGTTCCCCCCTAAGCCCAAGGACACCCCTGATGATCAGCCGCACCCCGAGGTGACCTGCGTGGTGGTGGACGTG
 AGCCACGAGAGCCCCGAGGTGAAGTTCACCTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCG
 CGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT
 AAGGAGTACAAGTGAAGGTGAGCAACAAGGCCCTGCCCGCCCCATCGAGAAGACCATCAGCAAGGCCAAGGGC
 15 CAGCCCCCGCAGCCCCAGGTGTACACCTGCCCCCAGCCGCGACGAGCTGACCAAGAACCAGGTGAGCCTGACC
 TGCCTGGTGAAGGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACCTAC
 AAGACCACCCCCCGTGTGGACAGCGACGGCAGCTTCTTCTGTACAGCAAGCTGACCGTGGACAAGAGCCGCG
 TGGCAGCAGGGCAACGTGTTTACGTGCGAGCGTGATGACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCCTG
 AGCCTGAGCCCCGGCTAACTCGAGAATCAACCTCTGGATTACAAAATTTGTGAAGATTGACTGGTATTCTTAAAC
 20 TATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTAAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCT
 TTCATTTTCTCCTTGTATAAATCCTGGTGTGCTGCTCTTTATGAGGAGTTGTGGCCCGTTGTGAGGCAACGT
 GCGTGGTGTGCACTGTGTTTGTGACGCAACCCCCACTGGTGGGGCATTGCCACCACCTGTGAGCTCCTTTCC
 GGGACTTTCGCTTTCCCCCTCCCTATTGCCAGCGGGAACCTATCGCCGCTGCTTGCCTTGCCTGCTGGACAGGG
 GCTCGGCTGTTGGGCACGTGACAATCCGTGGTGTGTTGTCGGGGAAATCATCGTCCCTTTCCTTGGCTGCTCGCTGT
 25 GTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCTTCC
 CGCGGCTGCTGCCGGCTCTGCGGCTCTTCCGCTCTTGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGG
 GCCGCTCCCCGCATCGATACCGTCTGACTCGCTGATCAGCCTGTGCTTCTAGTTGCCAGCCATCTGTTGTTTGC
 CCTCCCCCGTGCCTTCCCTTGACCCTGGAAGGTGCCACTCCCCTGCTCTTCCCTAATAAAATGAGGAAATTGCA
 TCGCATTGTCTGAGTAGGTGTATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAA
 30 GACAATAGCAGGCATGCTGGGATGCGGTGGGCTCTATGGGCGGCCGAGGAACCCCTAGTGATGGAGTTGGCCA
 CTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAGGTGCCCCGACGCGCGGCTTTGCCCGGG
 CGGCCTCAGTGAGCGAGCG

(SEQ ID NO: 49)

In some embodiments, the polynucleotide comprises or consists of the nucleotide sequence
 35 of SEQ ID NO: 50, 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.

CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCCGGGCGTGGGCGACCTTTGGTGCGCCCGGCTCAGTGAGC
 GAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCTTGGCGCGCCGAGTTCCGCGTTAC
 40 ATAACCTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTA
 TGTTCCCATAGTAACGCCAATAGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCCA
 CTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
 CTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGC
 TATTACCATGGTGTGCGGTTTGGCAGTATCAATGGGCGTGGATAGCGGTTTGACTCACGGGATTTCG
 45 AAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCTG
 TAACAACCTCCGCCCATTGACGCAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCG
 TTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGACTAGT
 GCCACCATGCGCCTGCTGGCCAAGATCATCTGCCTGATGCTGTGGGCCATCTGCGTGGCCAGCGACACCGGC
 CGCCCCCTTCGTGGAGATGTACAGCGAGATCCCCGAGATCATCCACATGACCGAGGGCCGCGAGCTGGTGATC
 50 CCTGCGCGTGACCGCCCCAACATCACCGTGACCCCTGAAGAAGTTCCTTGGACACCCTGATCCCCGAC
 GGCAAGCGCATCATCTGGGACAGCCGCAAGGGCTTCATCATCAGCAACGCCACCTACACCGGAGATCGGCCCTG
 CTGACCTGCGAGGGCCACCGTGAACGGCCACCTGTACAAGACCAACTACCTGACCCACCGCCAGACCAACACC
 ATCATCGACGTGGTGTGAGCCCCAGCCAGGCATCGAGCTGAGCGTGGGCGAGAAGCTGGTGTGAAGTGC
 ACCGCCCGCACCGAGCTGAACGTGGGCATCGACTTCAACTGGGAGTACCCAGCAGCAAGCACCAGCACAAAG
 55 AAGCTGGTGAACCGGACCTGAAGACCCAGAGCGGCAGCGAGATGAAGAAGTTCCTGAGCACCCCTGACCATC

GACGGCGTGACCCGACGACAGGGCGCTGTACACCTGCGCCGCCAGCAGCGGCGCTGATGACCAAGAAGAAC
 AGCACCTTCGTGCGCGTGACGAGAAAGGACAAGACCCACACCTGCCCCCCTGCCCCGCCCCGAGCTGCTG
 GGCGGCCCCAGCGTGTTCTGTTCCTGTTCCCTCCCAAGCCCAAGGACACCCTGATGATCAGCCGACCCCCGAGGTG
 5 ACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAG
 GTGCACAACGCCAAGACCAAGCCCCGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGGCGTGTGACC
 GTGCTGCACCAAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAAGGCCCTGCCCGCCCC
 ATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACCTGCCCCCAGCCGC
 GACGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCTGGTGAAGGGCTTCTACCCAGCGACATCGCCGTG
 10 GAGTGGGAGAGCAACGCCAGCCGAGCCGAGAACAACATAAGACCACCCCCCGTGTGAGACAGCGACGGCAGC
 TTCTTCTGTACAGCAAGCTGACCGTGGACAAGAGCCGCTGGCAGCAGGGCAACGTGTTCACTGACGCGTG
 ATGCACGAGGCCCCGACACAACCTACACCCAGAAGAGCCTGAGCCTGAGCCCCGGCTAACGAAGGAAACGA
 GGAAGCGGAGAAGCCAGACACAAACAGAAAATTGTGGCACCCTGAAACAGACTTTGAATTTTGACCTTCTC
 AAGTTGGCGGGAGACGTCGAGTCCAACCTGGGCCCCATGCGCCTCCTGGCCAAGATCATCTGCCTCATGCTG
 15 TGGGCCATCTGCGTGGCTGAGGACTGCAATGAGCTGCCGCCAGGAGGAACACAGAGATCCTGACAGGGAGC
 TGGTCTGACCAGACCTACCCTGAGGGCACCCAGCGATCTACAAGTGCCGCGCGGGCTACAGGAGCCTGGGG
 AACATCATCATGGTGTGTAGAAAGGGCGAATGGGTGGCCCTCAACCCCTGAGGAAGTGCCAGAAGCGGCC
 TGTGGCCACCCCGGGGACACACCTTCGGGACCTTCACCTGACCGCGGCAATGTGTTTGTGAGTACGGCGTG
 AAGGCTGTCTACACATGCAACGAGGGGTACCAGTGCTGGCGGAGATTAACTACCGGAGTGTGACACCGAT
 GGGTGGACCAACGACATTCCCCTCTGTGAGGTGGTCAAGTGTCTCCCGTGACAGCCCCAGAAAATGGCAAA
 20 ATCGTGAGCAGCGCCATGGAGCCTGACCGCGAATATCACTTTGGGCAGGCCGTGAGGTTTGTGTCAACTCG
 GGCTACAAAATTGAAGGTGATGAGGAGATGCACTGCAGCGATGATGGCTTCTGGTCCAAGGAGAAGCCCAA
 TGTGTGGAGATCTCTGCAAGTCTCCCGACGTGATCAACGGCAGCCCAATCAGCCAGAAGATTATTTACAAA
 GAGAAGCAGCGCTTCCAGTACAAGTGTAAATGGGCTATGATATTAGAGAGGGGAGATGCCGTCTGCACT
 GAGAGCGGCTGGAGACCACTGCCCTAGCTGCGAGGAAAAGAGTTGTGACAACCTTACATCCCAAATGGCGAC
 25 TACTCCCCCTGCGGATCAAACACCGGACCGGGGATGAAATCACCTATCAGTGCCGCAATGGATTCTACCCG
 GCCACCCGCGGCAACACCGCCAAATGCACCAGCACAGGCTGGATCCCCGCCCCCGCTGTACGCTGAAGCCT
 TGCGACTATCCAGACATCAAGCACGAGGCCTGTACCACGAAAACATGCGCGCGCCTTATTTCCCTGTGGCA
 GTGGGGAAGTACTACAGCTACTACTGCGACGAGCACTTCGAGACCCCTCTGGCTCCTACTGGGACCACATC
 CACTGCACACAGGACGGCTGGTCTCCAGCTGTGCCCTGCCCTGAGGAAATGCTACTTCCCCTACCTGGAGAAC
 30 GGATACACCAAGAACTATGGCCGCAAGTTCTGTGAGGCAAGAGCATCGATGTGGCTGCCACCTGGCTTAC
 GCCCTGCCCAAGGCCAGACAACCTGTGACCTGCATGGAGAATGGTTGGAGCCCCACCCCGCGCTGCATCCGG
 GTGTCTTACGCTCCTCGAGAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAAC
 TATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATG
 GCTTTTCATTTTCTCCTTGTATAAATCCTGGTTGCTGTCTTTATGAGGAGTTGTGGCCCGTTGTGAGG
 35 CAACGTGGCGTGGTGTGCACTGTGTTTGTGCTGACGCAACCCCACTGGTTGGGGCATTGCCACCACTGTGAG
 CTCCTTTCCGGGACTTTCGCTTTCCCTCCTTATTGCCACGGCGGAACATCATCGCCGCTGCCTTGGCCGC
 TGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTGTCGGGGAAATCATCGTCTTTTCT
 TGGCTGTGCGCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGTACGTCCCTTCGGCCCTCAAT
 40 CCAGCGGACCTTCTTCCCGCGGCTGTGCGGCTCTGCGGCTCTTCCGCGTCTTCCGCTTCGCCCTCAG
 ACGAGTCGGATCTCCCTTTGGGCGGCTCCCCGCATCGATACCGTCGACTCGCTGATCAGCCTGTGCCCTTCT
 AGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCGCTGCCTTCTTGACCTGGAAGGTGCCACTCCACTGTC
 CTTTCTTAATAAATGAGGAAATGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGTGGGGTG
 GGGCAGGACAGCAAGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGG
 45 GGCCGCAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGG
 GACCAAAGGTGCGCCGACGCGCGGGCTTTGCCCCGGCGGCTCAGTGAGCGAGCG

(SEQ ID NO: 50)

In some embodiments, the polynucleotide comprises or consists of the nucleotide sequence
 of SEQ ID NO: 51, 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
 50 thereto.

CGCTCGCTCACTGAGGCCGCCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTGCAGCCGCGCTCAGTGAGC
 GAGCGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTTCTGGCGCGCCCCATTGACGTCAATA
 ATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAA
 55 ACTGCCCACTTGGCAGTACATCAAGTGATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAA
 TGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTA

GTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCA
CCCCCAATTTTGTATTTATTTATTTTAAATTATTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGCGC
GCGCCAGCGGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGCGGAGAGGTGCGGCGGCAGCCAATCAG
AGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGGCGGGCGGGCGGGCCCTATAAAAAGCGAAGCGCG
5 CGGCGGGCGGGAGTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCCGCCGCTCGCGCGCCCGCCCC
CGGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAG
CGCTTGTTTAAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCT
TTGTGCGGGGGAGCGGCTCGGGGCTGTCCGCGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGG
10 GTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTCT
ACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATTGGATCCACTAGTGCC
ACCATGCGCCTCCTGGCCAAGATCATCTGCCTCATGCTGTGGCCCATCTGCGTGGCTGAGGACTGCAATGAG
CTGCGCGCCAGGAGGAACACAGAGATCCTGACAGGGAGCTGGTCTGACCAGACCTACCCTGAGGGCACCAG
GCGATCTACAAGTGCCGGCCGGGCTACAGGAGCCTGGGGAACATCATCATGGTGTGTAGAAAGGGCGAATGG
GTGGCCCTCAACCCCTGAGGAAGTGCCAGAAGCGGCCCTGTGGCCACCCCGGGGACACACCCTTCGGGACC
15 TTCACCTTGACCGGCGCAATGTGTTTGTGAGTACGGCGTGAAGGCTGTCTACACATGCAACGAGGGGTACCAG
CTGCTGGGCGAGATTAACCTACCGGAGTGAGTACACCGATGGTGAGCAACGACATTCCTCATCTGTGAGGTG
GTCAAGTGTCTCCCCGTGACAGCCCCAGAAAATGGCAAATCGTGAGCAGCGCCATGGAGCCTGACCGCGAA
TATCACTTTGGGCAGGCGGTGAGGTTTGTGTGCAACTCGGGCTACAAAATTGAAGGTGATGAGGAGATGCAC
TGCAGCGATGATGGCTTCTGGTCCAAGGAGAAGCCCAAATGTGTGGAGATCTCCTGCAAGTCTCCCGACGTG
20 ATCAACGGCAGCCCAATCAGCCAGAAGATTATTTACAAAGAGAACGAGCGCTTCCAGTACAAGTGTACATG
GGCTATGAGTATTACAGAGAGGGGAGATGCCGTCTGCACTGAGAGCGGCTGGAGACCCTGCCTAGCTGCGAG
GAAAAGAGTTGTGACAAACCTTACATCCCAAATGGCGACTACTCCCCTCTGCGGATCAACACCGGACCGGG
GATGAAATCACCTATCAGTGCCGCAATGGATTCTACCGGCCACCCGCGGCAACACCGCCAAATGCACCAGC
ACAGGCTGGATCCCCGCCCCCGCTGTACGCTGAAGCCTTGCGACTATCCAGACATCAAGCACGGAGGCTG
25 TACCACGAAAACATGCGGCGGCCCTTATTTCCCTGTGGCAGTGGGGAAGTACTACAGCTACTACTGCGACGAG
CACTTCGAGACCCCCCTCTGGCTCCTACTGGGACCACATCCACTGCACACAGGACGGCTGGTCTCCAGCTGTG
CCCTGCCTGAGGAAATGCTACTTCCCTACCTGGAGAACGGATACAACCAGAACTATGGCGCAAGTTCTGTG
CAGGGCAAGAGCATCGATGTGGCTGCCACCCTGGCTACGCCCTGCCCAAGGCCAGACAACCTGTGACCTGC
ATGGAGAATGGTTGGAGCCCCACCCGCGCTGCATCCGGGTGTCCTTCACGCTCCGAAGGAAACGAGGAAGC
30 GGAGAAGCCAGACACAAACAGAAAATTGTGGCACCGGTGAAACAGACTTTGAATTTTACCTTCTCAAGTTG
GCGGGAGACGTGAGTCCAACCTGGGCCCATGCGCCTGCTGGCCAAGATCATCTGCCTGATGCTGTGGGCC
ATCTGCGTGGCCAGCGACACCGGCGCCCCCTTCGTGGAGATGTACAGCGAGATCCCCGAGATCATCCACATG
ACCGAGGGCCGCGAGCTGGTGATCCCTGCCGCGTGACAGCCCCAACATCACCGTGACCTGAAGAAGTTC
CCCCTGGACACCCTGATCCCCGACGGCAAGCGCATCATCTGGGACAGCCGCAAGGGCTTCATCATCAGCAAC
35 GCCACTACAAGGAGATCGGCTGCTGACCTGCGAGGCCACCGTGAACGGCCACCTGTACAAGACCAACTAC
CTGACCCAGCCGACACAAACACCATCATCGACGTGGTGCTGAGCCCCAGCCACGGCATCGAGCTGAGCGTG
GGCGAGAAGCTGGTGTGAACCTGCACCGCCCGCACCGAGCTGAACGTGGGCATCGACTTCAACTGGGAGTAC
CCCAGCAGCAAGCACAGCACAAGAAGCTGGTGAACCGCGACCTGAAGACCCAGAGCGGCAGCGAGATGAAG
AAGTTCCTGAGCACCTTGACCATCGACGGCGTGACCCGCGAGCGACAGGGCTGTACACCTGCGCCGCCAGC
40 AGCGGCCCTGATGACCAAGAAGAAGACAGCACCTTCGTGCGCGTGACAGGAGAAGGACAAGACCCACACCTGCCCC
CCCTGCCCGCCGCCCCGAGCTGCTGGGCGGGCCCCAGCGTGTTCCTGTTCCCCCAGCCCAAGGACACCTTG
ATGATCAGCCGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAAGTTC
AACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGCGAGGAGCAGTACAACAGCACC
TACCGCGTGGTGAGCGTGTGACCGTGTGACACAGGACTGGCTGAACGGCAAGGAGTACAAGTGAAGGTG
45 AGCAACAAGGCCCTGCCCGCCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAGCCCCAG
GTGTACACCCTGCCCCCAGCCGCGACGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGC
TTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACATAAGAACACCCCCC
CCCGTGTGGACAGCGACGGCAGCTTCTTCTGTACAGCAAGCTGACCGTGGACAAGAGCCGCTGGCAGCAG
GGCAACGTGTTTCAGCTGCAGCGTGTGACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGAGCCTG
50 AGCCCCGCTAACTCGAGAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACATAT
GTTGCTCCTTTTACGCTATGTGGATACGCTGCTTAAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCT
TTCATTTTCTCCTCTTGTATAAATCCTGGTTAGTTCTTGCCACGGCGGAACTCATCGCCGCTGCCTTGCC
CGCTGCTGGACAGGGGCTCGGCTGTGGGCACTGACAATTCCTGGTGTGCCTTCTAGTTGCCAGCCATCTG
TTGTTTGGCCCTCCCCGCTGCCTTCCCTGACCTGGAAGGTGCCACTCCCCTGTCTTCTTAATAAAATG
55 AGGAAATTGCATCGCATTTGTCTGAGTAGGTGTATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGG
GGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGGCGGCCGAGGAACCCCT
AGTGATGGAGTTGGCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTGCCCCG
ACGCCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCG

(SEQ ID NO: 51)

In some embodiments, the polynucleotide comprises or consists of the nucleotide sequence of SEQ ID NO: 52, 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 CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCAGTGAGCGAG
CGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCTTGGCGCGCCCCATTGACGTCAATAATGACG
10 TATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCAC
TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGG
CATTATGCCAGTACATGACCTTATGGGACTTTCTTACTTTGGCAGTACATCTACGTATTAGTCATCGCTATTACC
ATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCAATTTTGTATTTA
15 TTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGCCAGGCGGGGCGGGGCGG
GGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAAATCAGAGCGGCGCGCTCCGAAAGTTTCTCT
TTTATGGCGAGGCGGGCGGGCGGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGGAGTCGCTGCGCGCTGC
CTTCGCCCCGTGCCCGCTCCGCGCGCGCTCGCGCGCCCCGCCCCGGCTCTGACTGACCGCGTTACTCCACAG
20 GTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAAATGACGGCTTGTCTTTCTTG
TGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGCTGTCCGCGGG
GGACGGCTGCCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGCGGCTCTAGAGCCTC
TGCTAACCATGTTTCATGCCTTCTTCTTTTCTTCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCA
25 TTTTGGCAAAGAATTGGATCCACTAGTGCCACCATGCGCCTGCTGGCCAAGATCATCTGCCTGATGCTGTGGGCC
ATCTGCGTGGCGAGCGACACCGGCCGCCCTTCGTGGAGATGTACAGCGAGATCCCCGAGATCATCCACATGACC
GAGGGCGCGGAGCTGGTGATCCCCCTGCCGCGTGACAGCCCCAACATCACCGTGACCTGAAGAAGTTCCCCCTG
GACACCTGATCCCCGACGGCAAGCGCATCATCTGGGACAGCCGAAGGGCTTCATCATCAGCAACGCCACCTAC
30 AAGGAGATCGCCTGCTGACCTGCGAGGCCACCGTGAAACGGCCACCTGTACAAGACCAACTACCTGACCCACCG
CAGACCAACACCATCATCGAGCTGGTGCTGAGCCCCAGCCACGGCATCGAGCTGAGCGTGGGCGAGAAGCTGGTG
CTGAAGTGCACCGCCCGCACCGAGCTGAACGTGGGCATCGACTTCAACTGGGAGTACCCAGCAGCAAGCACCAG
CACAAGAAGCTGGTGAACCGCGACCTGAAGACCCAGAGCGGCAGCGAGATGAAGAAGTTCTGAGCACCTGACC
ATCGACGGCGTGACCCGCGAGCGACAGGGCCTGTACACCTGCGCGCCAGCAGCGGCGCTGATGACCAAGAAGAAC
35 AGCACCTTCGTGCGCGTGCACGAGAAGGACAAGACCACACCTGCCCCCTGCCCCGCCCCGAGCTGCTGGGC
GGCCCCAGCGTGTTCTTGTTCCTGTTCCCCCCCCAAGCCAAAGGACACCTGATGATCAGCCGCACCCCCGAGGTGACCTGC
GTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAAC
GCCAAGACCAAGCCCCGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGAGCGTGTGACCGTGTGACCCAG
40 GACTGGCTGAACGGCAAGGAGTACAAGTGAAGGTGAGCAACAAGGCCCTGCCCGCCCCCATCGAGAAGACCATC
AGCAAGGCCAAGGGCCAGCCCCGCGAGCCCCAGGTGTACACCTGCCCCCAGCCGCGACGAGCTGACCAAGAAGAAC
CAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAG
CCCCGAGAACAACACAGACACCCCCCGTGTGCTGAGCAGCAGCGACGCGAGCTTCTCCTGTACAGCAAGCTGACC
45 GTGGACAAGAGCCGCTGGCAGCAGGGCAACGTGTTAGCTGCAGCGTGTGACGAGGCGCTGCACAACCACTAC
ACCCAGAAGAGCCTGAGCCTGAGCCCCGCTAACGAAGGAAACGAGGAAGCGGAGAAGCCAGACACAACAGAAA
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50 CCCATGCGCCTCCTGGCCAAGATCATCTGCCTCATGCTGTGGGCCATCTGCGTGGCTGAGGACTGCAATGAGCTG
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55 GCGGCAATGTGTTTGTAGTACGGCGTGAAGGCTGTCTACACATGCAACGAGGGGTACCAGCTGCTGGGCGAGATT
AACTACCGGGAGTGACACCGATGGGTGGACCAACGACATTCCCATCTGTGAGGTGGTCAAGTGTCTCCCCGTG
ACAGCCCCAGAAAATGGCAAAATCGTGAGCAGCGCCATGGAGCCTGACCGCGAATATCACTTTGGGCAGGCCGTG
AGGTTTGTGTGCAACTCGGGCTACAAAATGAAGGTGATGAGGAGATGCACTGCAGCGATGATGGCTTCTGGTCC
AAGGAGAAGCCCAATGTGTGGAGATCTCCTGCAAGTCTCCGACGTGATCAACGGCAGCCCCAATCAGCCAGAAG
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50 GTCTGCACTGAGAGCGGCTGGAGACCACTGCCTAGCTGCGAGGAAAAGAGTTGTGACAACCTTACATCCCAAT
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CCGGCCACCCGCGGCAACACCGCCAAATGCACAGCAGGCTGGATCCCCGCCCCCGCTGTACGCTGAAGCCT
TGCGACTATCCAGACATCAAGCAGGAGCCTGTACCAAGAAAACATGCGGCGGCTTATTTCCCTGTGGCAGTG
GGGAAGTACTACAGCTACTACTGCGACGAGCACTTCGAGACCCCTCTGGCTCCTACTGGGACCACATCCACTGC
55 ACACAGGACGGCTGGTCTCCAGCTGTGCCCTGCCTGAGGAAATGCTACTTCCCCTACCTGGAGAACGGATACAAC
CAGAACTATGCCGCAAGTTCTGTGAGGGCAAGAGCATCGATGTGGCTGCCACCTGGCTACGCCCTGCCAAG
GCCAGACAACTGTGACCTGCATGGAGAATGGTTGGAGCCCCACCCGCGCTGCATCCGGGTGTCCTTACGCTC

CTCGAGAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACG
CTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTG
TATAAATCCTGGTTAGTTCTTGCCACGGCGGAACATCATCGCCGCTGCCTTGCCCGCTGCTGGACAGGGGCTCGG
CTGTTGGGCACTGACAATCCGTGGTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTGCCCCCTCCCCCGTGCCTT
5 CCTTGACCCCTGGAAGGTGCCACTCCCCTGTCTTTCTAATAAAATGAGGAAATTCATCGCATTTGTCTGAGTA
GGTGTCAATTCATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATG
CTGGGGATGCGGTGGGCTCTATGGGCGGCCGAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGC
TCGCTCGCTCACTGAGGCCGGGCGACCAAGGTGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGA
GCG

10 (SEQ ID NO: 52)

In some embodiments, the polynucleotide comprises or consists of the nucleotide sequence of SEQ ID NO: 53, 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.

15 CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCCGGGCGACCTTTGGTCGCCCCGGCCTCAGTGAGCGAG
CGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTTCTTGGCGCGCCCCATTGACGTCAATAATGACG
TATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCAC
TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGG
CATTATGCCCAGTACATGACCTTATGGGACTTTCTTACTTTGGCAGTACATCTACGTATTAGTCATCGCTATTACC
20 ATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTA
TTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGGGCGCGCCAGGCGGGGCGGGGCGG
GGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCGAGCCAATCAGAGCGCGCGCTCCGAAAGTTTCCT
TTTATGGCGAGGCGGGCGGGCGGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGGAGTCGCTGCGCGCTGC
CTTCGCCCCGTGCCCGCTCCGCCCGCGCTCGCGCGCGCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAG
25 GTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTCTTTCTCTG
TGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGCTGTCCGCGGGG
GGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTC
TGCTAACCATGTTTCATGCCTTCTTCTTTTCTACAGCTCCTGGGCAACGTGCTGTTATTGTCTGTCTCATCA
TTTTTGGCAAAGAAATTGGATCCACTAGTGCCACCATGCGCCTCCTGGCCAAGATCATCTGCCTCATGCTGTGGGCC
30 ATCTGCGTGGCTGAGGACTGCAATGAGCTGCCGCCAGGAGGAACACAGAGATCCTGACAGGGAGCTGGTCTGAC
CAGACCTACCTGAGGGCACCCAGGCGATCTACAAGTGCCGGCGGGCTACAGGAGCCTGGGGAACATCATCATG
GTGTGTAGAAAGGGCGAATGGGTGGCCCTCAACCCCTGAGGAAGTGCCAGAAGCGGCCCTGTGGCCACCCCGGG
GACACACCCCTTCGGGACCTTACCCTGACCGGCGGCAATGTGTTTGTGACGTACGGCGTGAAGGCTGTCTACACATG
AACGAGGGGTACCAGCTGCTGGGCGAGATTAACCTACGGGAGTGTGACACCGATGGGTGGACCAACGACATTCCC
35 ATCTGTGAGGTGGTCAAGTGTCTCCCCGTGACAGCCCCAGAAAATGGCAAATCGTGAGCAGCGCCATGGAGCCT
GACCGCGAATATCACTTTGGGCGAGCCGTGAGGTTTGTGTGCAACTCGGGCTACAAAATTGAAGGTGATGAGGAG
ATGCACTGCAGCGATGATGGCTTCTGGTCCAAGGAGAGGCCCAAATGTGTGGAGATCTCCTGCAAGTCTCCCGAC
GTGATCAACGGCAGCCCAATCAGCCAGAAGATTATTTACAAAGAGAACGAGCGCTTCCAGTACAAGTGTAAACATG
GGCTATGAGTATTCAGAGAGGGGAGATGCCGTCTGCATGAGAGCGGCTGGAGACCACTGCCCTAGCTGCCAGGAA
40 AAGAGTTGTGACAACCCTTACATCCCAAATGGGCACTACTCCCTCTGCGGATCAAACACCGGACCGGGGATGAA
ATCACCTATCAGTGCCGCAATGGATTCTACCCGGCCACCCGCGGCAACACCGCCAAATGCACGACACAGGCTGG
ATCCCCGCCCCCGCTGTACGCTGAAGCCTTGCAGCTATCCAGACATCAAGCAGGAGGCCTGTACCACGAAAC
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45 TACTTCCCCTACTTGGAGAACGGATACACACAGAAGTATGGCCGCAAGTTCGTGCAGGGCAAGAGCATCGATGTG
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CCGCGCTGCATCCGGGTGTCTTACGCTCCGAAGGAAACGAGGAAGCGGAGAAGCCAGACACAAACAGAAAATT
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50 GTGGAGATGTACAGCGAGATCCCCGAGATCATCCACATGACCGAGGGCGCGAGCTGGTGATCCCCTGCCCGCTG
ACCAACCCCAACATACCGTGACCCCTGAAGAAGTTCCCCCTGGACACCCCTGATCCCCGACGGCAAGCGCATCATC
TGGGACAGCCGCAAGGGCTTCATCATCAGCAACGCCACCTACAAGGAGATCGGCCTGCTGACCTGCGAGGGCCACC
GTGAACGGCCACCTGTACAAGACCAACTACCTGACCAACCGCCAGACCAACACCATCATCGACGTGGTGCTGAGC
CCCAGCCACGGCATCGAGCTGAGCGTGGGCGAGAAGCTGGTGCTGAACTGCACCGCCCGCACCGAGCTGAACGTG
55 GGCATCGACTTCAACTGGGAGTACCCAGCAGCAAGCACCAGCACAGAAGCTGGTGAACCGCGACCTGAAGACC

CAGAGCGGCAGCGAGATGAAGAAGTTCCTGAGCACCTGACCATCGACGGCGTGACCCGAGCGACCAGGGCCTG
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ACCCACACCTGCCCCCCTGCCCCGCCCCGAGCTGCTGGGCGGCCCCAGCGTGTTCCTGTTCCCCCAAGCCC
AAGGACACCTGATGATCAGCCGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCGAG
5 GTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGCGAGGAGCAGTACAAC
AGCACCTACCGCGTGGTGGAGCTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAG
GTGAGCAACAAGGCCCTGCCGCCCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCCGCGAGCCCCAG
GTGTACACCTGCCCCCAGCCGCGACGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTC
TACCCACAGCGACATCGCCGTGGAGTGGGAGAGCAACGCCAGCCGAGCCGAGAACAATAACAAGACCACCCCCCCTG
10 CTGGACAGCGACGGCAGCTTCTTCTGTACAGCAAGCTGACCGTGGACAAGAGCCGCTGGCAGCAGGGCAACGTG
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CTCGAGGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTCCCCCTCCCCGTGCCCTTCTTGACCCCTGGAAGGTGCC
ACTCCCACTGTCCTTCTTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCTTCTATTCTGGGG
GGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCT
15 ATGGGCGGCGCAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCG
GGCGACCAAGGTGCGCCGACGCCCGGGCTTTGCCCGGGCGGCCCTCAGTGAGCGAGCG

(SEQ ID NO: 53)

In some embodiments, the polynucleotide comprises or consists of the nucleotide sequence
of SEQ ID NO: 54, or a nucleotide sequence that has at least 75%, 80%, 85%, 86%, 87%,
20 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity
thereto.

CGCTCGCTCACTGAGGCCGCGCGGCAAGCCCCGGGCGTGGGGCAGCTTTGGTGGCGCGCCCTCAGTGAGCGAG
CGAGCGCGCAGAGAGGGAGTGGCCAATCCATCACTAGGGGTTCTGGCGCGCCCCATTGACGTCAATAATGACG
25 TATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCAC
TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCCTGG
CATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACC
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30 GCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGCGCGCTCCGAAAGTTTCCT
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CTTCGCCCCGTGCCCCGTCCGCGCGCGCCTCGCGCGCCCCCGGCTCTGACTGACCGCGTTACTCCCACAG
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TGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGAGCGGCTCGGGGCTGTCCGCGGGG
35 GGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTC
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GAGGGCCGCGAGCTGGTGATCCCCCTGCCGCGTGACCAAGCCCCAACATCACCGTGACCTGAAGAAGTTCCTTGA
40 GACACCCTGATCCCCGACGGAAGCGCATCATCTGGGACAGCCGCAAGGGCTTCATCATCAGCAACGCCACCTAC
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CACAAGAAGCTGGTGAACCGCGACCTGAAGACCCAGAGCGGCGAGGATGAAGAAGTTCCTGAGCAACCTGACC
45 ATCGACGGCTGACCCGACGCGACAGGGCCTGTACACCTGCGCGCCAGCAGCGGCTGATGACCAAGAAGAAC
AGCACCTTCGTGCGCGTGACGAGAAGGACAAGACCCACACCTGCCCCCCTGCCCCGCCCCGAGCTGCTGGGC
GGCCCCAGCGTGTTCCTGTTCCCCCAGGCCAAGGACACCCTGATGATCAGCCGACCCCCGAGGTGACCTGC
GTGGTGGTGGACGTGAGCCACGAGGACCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAAC
GCCAAGACCAAGCCCCGCGAGGAGCAGTACAACAGCACCTACCGCGTGGTGGAGCTGCTGACCGTGTGACCCAG
50 GACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGAGCAACAAGGCCCTGCCCCCCCCATCGAGAAGACCATC
AGCAAGGCCAAGGGCCAGCCCCGCGAGGCCACAGGTGTACACCTGCCCCCAGCCGCGACGAGCTGACCAAGAAC
CAGGTGAGCCTGACCTGCCTGGTGAAGGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAG
CCCGAGAACAATAACAAGACCACCCCCCGTGTGGACAGCGACGGCAGCTTCTTCTGTACAGCAAGCTGACC
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55 ACCCAGAAGAGCTGAGCCTGAGCCCCGCTAACGAAGGAAACGAGGAAGCGGAGAAGCCAGACACAACAGAAA

ATGTGGCACCGGTGAAACAGACTTTGAATTTTGACCTTCTCAAGTTGGCGGGAGACGTCGAGTCCAACCCTGGG
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 CCGCCCAGGAGGAACACAGAGATCCTGACAGGGAGCTGGTCTGACCAGACCTACCCTGAGGGCAGCCAGGCGATC
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 5 AACCCCTGAGGAAGTGCCAGAAGCGGCCCTGTGGCCACCCCGGGGACACACCCTTCGGGACCTTCACCTGACC
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 AGTTTGTGTGCAACTCGGGCTACAAAATGAAGGTGATGAGGAGATGCACTGCAGCGATGATGGCTTCTGGTCC
 10 AAGGAGAAGCCCAATGTGTGGAGATCTCCTGCAAGTCTCCCGACGTGATCAACGGCAGCCCAATCAGCCAGAAG
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 GGCGACTACTCCCTCTGCGGATCAAACACCGGACCGGGGATGAAATCACCTATCAGTGCCGCAATGGATTCTAC
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 15 TCGCACTATCCAGACATCAAGCACGGAGCGCTGTACCACGAAAACATGCGGCGGCCCTATTTCCCTGTGGCAGTG
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 ACACAGGACGGCTGGTCTCCAGCTGTGCCCTGCCTGAGGAAATGCTACTTCCCTACCTGGAGAACGGATACAAC
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 20 CTCGAGGTGCCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCC
 ACTCCCACTGTCCTTTCCCTAATAAAATGAGGAAATGCATCGCATGTCTGAGTAGGTGTCATTCTATTCTGGGG
 GGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCT
 ATGGGCGGCCGAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCG
 GCGGACCAAGGTGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCCTCAGTGAGCGAGCG

25 (SEQ ID NO: 54)

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).

- 30 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

- 35 The retina is the multi-layered tissue, 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 distal to the retinal pigment epithelium.

Neurosensory retina and photoreceptor cells

- 40 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 inner and outer segments of the rods and cones.

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.

Rod cells are distributed mainly across the peripheral 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 and are responsible for colour vision. There are on average about 6-7 million cone cells in a normal human retina.

Retinal pigment epithelium

The retinal pigment epithelium (RPE) is a pigmented layer of cells located immediately to the outside of the neurosensory retina, adjacent to the photoreceptor outer segments. The RPE separates the neurosensory retina from the choroidal vasculature and has functions such as recycling the visual pigment from the photoreceptor outer segments. 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.

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)

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 µm), medium (63-124 µm) and large (>124µm). 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.

AMD is classified into “dry” and “wet” (exudative or neovascular) forms. Dry AMD is typically characterised by progressive cell loss 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 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.

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.

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 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 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.

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.

- 5 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.

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

- 10 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 untreated, diabetic retinopathy can cause blindness.

- 15 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.

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.

- 20 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
25 and types of diabetic retinopathy.

Vectors

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.

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

5 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.

10 The polynucleotide comprising nucleotide sequences encoding (i) an anti-VEGF entity and (ii) a negative complement regulator as provided by the present invention may be suitable for packaging in AAV. AAV is known to be capable of packaging genomes of up to 5.2 kb in size (Dong, J.-Y. et al. (1996) Human Gene Therapy 7: 2101-2112).

15 As such, the nucleotide sequences encoding the anti-VEGF entity and the negative complement regulator may be selected such that the combination is suitable for packaging in AAV. In certain embodiments, functional fragments of an anti-VEGF entity and/or a negative complement regulator (such as those described herein) may be used to ensure that the polynucleotide is suitable for packaging in AAV.

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 present polynucleotide is less than or equal to 4.7 kb.

20 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.

25 The AAV genome may be in single-stranded form, either positive or negative-sense, or alternatively in double-stranded form.

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.

30 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
5 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, Rec3, Rh10, DJ or Anc65 AAV vector particle.

In some embodiments, the AAV vector particle may comprise an AAV1, AAV2, AAV3, AAV4,
10 AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 or AAV11 capsid.

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 preferred embodiments, the AAV may be an AAV8 serotype.

15 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
20 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, AY629583,
25 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
30 can be traced back to a common ancestor, and includes all descendants thereof. 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.

5 The skilled person can select an appropriate serotype, clade, clone or isolate of AAV for use 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).

10 The AAV serotype determines the tissue specificity of infection (or tropism) of an AAV. 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.

15 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 anti-VEGF entity and negative complement regulator. The AAV
20 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.

25 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.

30 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 vectors which between them comprise 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.

- 5 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
10 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
15 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 anti-VEGF entity and negative complement regulator 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
20 expression of the transgene in vivo.
25

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
30 into the host cell genome. Additionally, reducing the size of the AAV genome 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: the replication (rep) and capsid (cap) genes. However, in some embodiments, derivatives may

additionally include one or more rep and/or 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.

- 5 Where a derivative comprises capsid proteins i.e. VP1, VP2 and/or VP3, the derivative may 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).
- 10 Chimeric, shuffled or capsid-modified derivatives will be typically selected to provide one or 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 or AAV8. Increased
- 15 efficiency of gene delivery may be effected by improved receptor or co-receptor binding at 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
- 20 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

25 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

30 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 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 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- and/or C-terminus of a capsid coding sequence. This may be done, for example, to confer or change tropism towards specific cell receptors.

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 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 and Dalkara *et al.* (Science, Translational Medicine, 2013; 5(189)).

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 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 an AAV genome or derivative thereof and a sequence encoding the anti-VEGF entity and negative complement regulator transgenes 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 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 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.

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

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.

In some embodiments, the polynucleotide comprises a 5' AAV ITR with the nucleotide sequence of SEQ ID NO: 55, 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.

CGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCAGTGAGC
GAGCGAGCGCGCAGAGAGGGAGTGGCCAACCTCCATCACTAGGGGTTTCCT

SEQ ID NO: 55

In some embodiments, the polynucleotide further comprises the nucleotide sequence of SEQ ID NO: 56, 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.

TGTAGTTAATGATTAACCCGCCATGCTACTTATCTACGTAGCCATGCTCTAGGTACC

SEQ ID NO: 56

30 In some embodiments, the polynucleotide comprises a 5' AAV ITR with the nucleotide sequence of SEQ ID NO: 65, 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 aggaaccacctagtgatggagttggccactccctctctgcgcgctcgctcgctcactgagggcgccgggcaa
agcccgggcgctcgggcgacctttggcgcccgccctcagtgagcgagcgagcgcgagagagggagtgggca
a

SEQ ID NO: 65

10 In some embodiments, the polynucleotide comprises a 3' AAV ITR with the nucleotide sequence of SEQ ID NO: 57, 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
AGGTCGCCCCGACGCCCGGGCTTTGCCCGGGCGCCTCAGTGAGCGAGCG

SEQ ID NO: 57

15 In some embodiments, the polynucleotide further comprises the nucleotide sequence of SEQ ID NO: 58, 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.

CTTCTGAGCGGAAAGAACCAGCTGGGGCTCGACTAGAGCATGGCTACGTAGATAAGTAGCATGGCGGGTTA
ATCATTA ACTACA

20 SEQ ID NO: 58

In some embodiments, the polynucleotide comprises a 3' AAV ITR with the nucleotide sequence of SEQ ID NO: 66, 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.

25 aggaaccacctagtgatggagttggccactccctctctgcgcgctcgctcgctcactgagggcgggcgaccaa
aggctgccccgacgcccgggctttgccccggcgccctcagtgagcgagcgagcgcgagagagggagtgggca
a

SEQ ID NO: 66

Promoters and regulatory sequences

30 The polynucleotide or vector of the invention may also include elements allowing for the expression of the anti-VEGF entity and/or negative complement regulator 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 or CAG promoter, optionally wherein the CMV or CAG promoter is upstream of the nucleotide sequences encoding the anti-VEGF entity and/or the negative complement regulator; (b) a WPRE regulatory element, optionally wherein the WPRE regulatory element is downstream of the nucleotide sequences encoding the anti-VEGF entity and/or the negative complement regulator; 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 anti-VEGF entity and/or the negative complement regulator.

In some embodiments, the polynucleotide or vector comprises:

- (a) a 5' AAV ITR;
- (b) a promoter, optionally a CMV promoter;
- (c) a nucleotide sequence encoding a negative complement regulator;
- (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 an anti-VEGF entity, preferably aflibercept;
- (f) optionally, a WPRE regulatory element, optionally a WPRE3 regulatory element;
- (g) a poly-A signal, optionally a Bovine Growth Hormone poly-A signal; and
- (h) a 3' AAV ITR.

The negative complement regulator may be selected from CFI and FHL-1.

Elements (c) and (e) may be in the reciprocal positions.

As such, in some embodiments, the polynucleotide or vector comprises:

- (a) a 5' AAV ITR;
- (b) a promoter, optionally a CMV promoter;
- (c) a nucleotide sequence encoding an anti-VEGF entity, preferably aflibercept;
- (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 a negative complement regulator;

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

5 In some embodiments, the polynucleotide or vector comprises:

- (a) a 5' AAV ITR;
- (b) a promoter, optionally a CAG promoter;
- (c) a nucleotide sequence encoding a negative complement regulator, preferably FHL-1;
- 10 (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 an anti-VEGF entity, preferably aflibercept;
- (f) optionally, a WPRE regulatory element, further optionally a WPRE3 regulatory element;
- 15 (g) a poly-A signal, optionally a Bovine Growth Hormone poly-A signal; and
- (h) a 3' AAV ITR.

Elements (c) and (e) may be in the reciprocal positions.

As such, in some embodiments, the polynucleotide or vector comprises:

- 20 (a) a 5' AAV ITR;
- (b) a promoter, optionally a CAG promoter;
- (c) a nucleotide sequence encoding an anti-VEGF entity, preferably aflibercept;
- (d) a linker, optionally wherein the linker comprises or is defined by a Furin cleavage site, GSG, 11a1D and an F2A sequence;
- 25 (e) a nucleotide sequence encoding a negative complement regulator, preferably FHL-1;
- (f) optionally, a WPRE regulatory element, further optionally a WPRE3 regulatory element;
- 30 (g) a poly-A signal, optionally a Bovine Growth Hormone poly-A signal; and
- (h) a 3' AAV ITR.

The polynucleotide may comprise (a)-(h) in order, from 5' to 3'. Suitably, (a) is at the 5' end of the polynucleotide and (h) is at the 3' end of the polynucleotide. Suitably, (b) is upstream of

(c)-(g). Suitably, (f) is downstream of (b)-(e). Suitably, (g) is downstream of (b)-(f). Suitably, (c) is upstream of (d) and (e) is downstream of (d).

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. The promoter may also enable expression, in particular tissue- or cell-specific expression, in ganglion cells, Mueller cells, photoreceptor cells, retinal pigment epithelium cells, and/or endothelial cells.

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.

An example CMV promoter sequence is:

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GGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCGCCATTGAC
GTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTT
ACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGA
CGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGA
CTCACGGGGATTTCCAAGTCTCCACCCCATGACGTCAATGGGAGTTTGTGTTTGGCACCAAAATCAACGGGA
CTTTCCAAAATGTCGTAACAACTCCGCCCATGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTA
TATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAG
AAGACACCG

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(SEQ ID NO: 59)

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: 59. Preferably, wherein the nucleotide sequence substantially retains the functional activity of the promoter represented by SEQ ID NO: 59.

In other embodiments, the polynucleotide or vector comprises a promoter with the nucleotide sequence of SEQ ID NO: 60.

An example CAG promoter sequence is:

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5      CCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTG
      GAGTATTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGAC
      GTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAG
      TACATCTACGTATTAGTCATCGCTATTACCATGGTTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATC
      TCCCCCCCCTCCCCACCCCAATTTGTATTTATTTATTTTAAATTATTTGTGCAGCGATGGGGGCGGGG
10     GGGGGGGGGGGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCG
      GCGGCAGCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCCCTAT
      AAAAAGCGAAGCGCGCGGCGGGGCGGAGTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCC
      TCGCGCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCT
      CCGGGCTGTAATTAGCGCTTGGTTAATGACGGCTTGTTCCTTTCTGTGGCTGCGTGAAAGCCTTGAGGGG
15     CTCCGGGAGGGCCCTTGTGCGGGGGGAGCGGCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGG
      ACGGGGACGGGCGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGC
      CTTCTTCTTTTCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATT
      GGATCC (SEQ ID NO: 60)

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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: 60. Preferably, wherein the nucleotide sequence substantially retains the functional activity of the promoter represented by SEQ ID NO: 60.

In other embodiments, the polynucleotide or vector comprises a promoter with the nucleotide sequence of SEQ ID NO: 60.

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:

5 GTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACT
 CCCACTGTCCTTTCTAATAAAATGAGGAAATTGCATCGCATGTCTGAGTAGGTGTCATTCTATTCTGGGG
 GGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGC
 TCTATGG

(SEQ ID NO: 61)

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

10 TCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCCTT
 GACCCTGGAAGGTGCCACTCCCACTGTCCTTTCTAATAAAATGAGGAAATTGCATCGCATGTCTGAGTAG
 GTGTCATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCA
 TGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCTGGGG

(SEQ ID NO: 62)

15 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: 61 or 62. Preferably, wherein the nucleotide sequence substantially
 retains the functional activity of the polyadenylation signal represented by SEQ ID NO: 61 or
 62.

In other embodiments, the polynucleotide or vector comprises a polyadenylation signal with
 the nucleotide sequence of SEQ ID NO: 61 or 62.

20 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 an AAV vector of the invention
 is the woodchuck hepatitis post-transcriptional regulatory element (WPRE) or a variant
 25 thereof.

An example WPRE is:

30 ATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACATATGTTGCTCCTTTACGCTAT
 GTGGATACGCTGCTTAAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTCTCCTCCTTGT
 ATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTGAGGCAACGTGGCGTGGTGTGCACTG
 TGTGTTGCTGACGCAACCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTTCGCTT
 TCCCCCTCCCTATTGCCACGGCGGAACATCATCGCCGCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGT
 TGGGCACTGACAATCCGTGGTGTGTCGGGGAAATCATCGTCCTTTCTTGGCTGCTCGCCTGTGTTGCCA
 CCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCTTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCG
 35 GCCTGCTGCCGGCTCTGCGGCCTCTCCGCGCTTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGG
 CCGCCTCCCCGC

(SEQ ID NO: 63)

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.

- 5 An example WPRE3 sequence is:

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AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTA
TGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTG
TATAAATCCTGGTTAGTTCTTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCT
CGGCTGTTGGGCACTGACAATTCCGTGGT
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- 10 (SEQ ID NO: 64)

- 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: 63 or 64. Preferably, wherein the nucleotide sequence substantially retains the functional activity of the post-transcriptional regulatory element represented by SEQ ID NO: 63 or 64.

The WPRE may be derived from wild type or modified woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) sequences

In other embodiments, the polynucleotide or vector comprises a post-transcriptional regulatory element with the nucleotide sequence of SEQ ID NO: 63 or 64.

- 20 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

- 25 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

- 30 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 .

- 5 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
10 lifetime of a subject.

Subretinal injection

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.

- 15 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".

- 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
20 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 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.

- 25 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.

- Unless damage to the retina occurs during the injection, and as long as a sufficiently small
30 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.

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

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 .
10 Larger volumes may increase the risk of stretching the retina, while smaller volumes may be difficult to see.

Two-step subretinal injection

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
15 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 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
20 second step.

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

- (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
25 vector; and
- (b) administering a medicament composition by subretinal injection into the bleb formed by step (a), wherein the medicament comprises the product, polynucleotide or vector.

The volume of solution injected in step (a) to at least partially detach the retina may be, for
30 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 .

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
 5 in step (b) is 100 μL . Larger volumes may increase the risk of stretching the retina, 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)
 10 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
 15 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
 20 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

25 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
 30 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
5 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

10 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
15 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
20 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
25 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
30 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.

- 5 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.

- 10 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.

- 15 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

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

- 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.

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.

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 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 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.

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 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 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). 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).

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 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 part of the sequence comparison and generates a numerical result.

“Fragments” 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 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 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.

5 Aspects of the Invention

Aspects of the invention are defined in the following numbered paragraphs (paras):

1. A product comprising (i) an anti-VEGF entity; and (ii) a negative complement regulator, or nucleotide sequences encoding therefor, as a combined preparation for simultaneous, separate or sequential use in therapy.
- 10 2. A product for use according to para 1 wherein the disease to be treated or prevented is an ocular disorder.
3. A product for use according to para 2 wherein the ocular disorder is a complement-mediated disorder of the eye.
- 15 4. The product for use according to para 3, wherein the complement-mediated disorder is age-related macular degeneration (AMD), diabetic retinopathy, glaucoma, Stargardt's disease, central serous chorioretinopathy, retinitis pigmentosa, polypoidal choroidal vasculopathy, diabetic macular edema, branch retinal vein occlusion or uveitis, preferably AMD.
5. The product for use according to para 4, wherein the AMD is wet AMD and/or dry AMD.
- 20 6. The product for use according to para 4, wherein the AMD is wet AMD and the use further prevents and/or treats onset of dry AMD in said subject.
7. The product for use according to any of paras 1 to 6 wherein the product comprises a polynucleotide comprising nucleotide sequences encoding (i) an anti-VEGF entity and (ii) a negative complement regulator.
- 25 8. An isolated polynucleotide comprising nucleotide sequences encoding (i) an anti-VEGF entity and (ii) a negative complement regulator.
9. A product for use according to para 7 or an isolated polynucleotide according to para 8 wherein the anti-VEGF entity is selected from an Ig fusion protein, an antibody, a polypeptide, a peptide, a non-antibody scaffold, an antisense oligonucleotide, siRNA, shRNA, CRISPR-guide strand and an aptamer.
- 30

10. A product for use or an isolated polynucleotide according to para 9 wherein the anti-VEGF entity is selected from aflibercept, ranibizumab, bevacizumab and pegaptanib.
11. An isolated polynucleotide according to para 10 wherein the anti-VEGF entity
5 aflibercept, for example wherein the aflibercept is encoded by a polynucleotide sequence comprising any one of SEQ ID NO: 3 to 11.
12. A product for use or an isolated polynucleotide according to any preceding para wherein the negative complement regulator is selected from Complement Factor I (CFI), Complement Factor H Like Protein 1 (FHL1), Complement Factor H (CFH),
10 Complement receptor type 1 (CR1), Membrane Cofactor Protein (MCP), Complement decay-accelerating factor (DAF), MAC-inhibitory protein (MAC-IP), C1-inhibitor, anaphylatoxins inhibitor, C4b binding protein (C4BP), clusterin, vitronectin or a variant or fragment thereof.
13. A product for use or an isolated polynucleotide according to para 12 wherein the
15 negative complement regulator is selected from CFI and FHL1 or a variant or fragment thereof.
14. The product for use or isolated polynucleotide according to any of paras 7 to 13, wherein the polynucleotide further comprises nucleotide sequences encoding:
 - (a) a CMV or CAG promoter, optionally wherein the CMV or CAG promoter is upstream of the nucleotide sequences encoding the (i) and (ii);
 - 20 (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
25 encoding the (i) and (ii).
15. The product for use or isolated polynucleotide according to any of paras 7 to 14, wherein the polynucleotide further comprises one or more adeno-associated virus (AAV) inverted terminal repeats (ITRs).
16. The product for use or isolated polynucleotide according to any of paras 7 to 15,
30 wherein the polynucleotide comprises an AAV ITR at its 5' end and an AAV ITR at its 3' end.

17. The product for use or isolated polynucleotide according to any of paras 7 to 16, wherein the polynucleotide comprises:
- (a) a 5' AAV ITR;
 - (b) a CMV or CAG promoter;
 - 5 (c) a nucleotide sequence encoding an anti-VEGF entity;
 - (d) a linker, optionally wherein the linker is comprises or is defined by a Furin cleavage site, GSG, 11aa1D sequence or an F2A sequence;
 - (e) a nucleotide sequence encoding a negative complement regulator;
 - 10 (f) optionally, a WPRE regulatory element, further 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.
18. The product for use or isolated polynucleotide according to any of paras 7 to 17, wherein the AAV ITRs are AAV2 ITRs.
- 15 19. The product for use or isolated polynucleotide according to any of paras 7 to 18, wherein the nucleotide sequences encoding the anti-VEGF entity and the negative complement regulator are codon optimised.
20. The product for use or isolated polynucleotide according to any of paras 7 to 19, wherein the nucleotide sequence encoding aflibercept has at least 75% sequence identity to SEQ ID NO: 11.
- 20 21. The product for use or isolated polynucleotide according to any of paras 7 to 20, wherein the nucleotide sequence encoding CFI has at least 75% sequence identity to SEQ ID NO: 35 or 36 or the nucleotide sequence encoding FHL-1 has at least 75% sequence identity to SEQ ID NO: 41.
- 25 22. The product for use or isolated polynucleotide according to any of paras 7 to 20, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 45 to 54, or a nucleotide sequence that has at least 75% sequence identity thereto.

23. The product for use or isolated polynucleotide according to any of paras 7 to 22, wherein the polynucleotide is less than or equal to 4.7 kb.
24. A vector comprising the polynucleotide of any one of paras 8-23.
25. The vector of para 24, wherein the vector is an adeno-associated viral (AAV) vector.
- 5 26. The vector of para 24 or 25, wherein the vector is in the form of a viral vector particle.
27. The vector of para 26, wherein the AAV vector particle comprises an AAV2 or AAV8 genome, and AAV2 or AAV8 capsid proteins.
28. A cell comprising the polynucleotide of any one of paras 7 to 22.
29. A cell transduced with the vector of any one of paras 24-27.
- 10 30. A pharmaceutical composition comprising the isolated polynucleotide, vector or cell of any one of paras 8-29 in combination with a pharmaceutically acceptable carrier, diluent or excipient.
31. The isolated polynucleotide, vector or cell of any one of paras 8-29 for use in therapy.
32. The isolated polynucleotide, vector or cell of any one of paras 8-29 for use in treating
15 or preventing a complement-mediated disorder of the eye.
33. The isolated polynucleotide, vector or cell for use according to para 32, wherein the complement-mediated disorder is age-related macular degeneration (AMD), diabetic retinopathy, glaucoma, Stargardt's disease, central serous chorioretinopathy, retinitis pigmentosa, polypoidal choroidal vasculopathy, diabetic macular edema, branch
20 retinal vein occlusion or uveitis, preferably AMD.
34. The isolated polynucleotide, vector or cell for use according to para 32, wherein the AMD is wet AMD and/or dry AMD.
35. The isolated polynucleotide, vector or cell for use according to para 33, wherein the AMD is wet AMD and the use further prevents and/or treats onset of dry AMD in said
25 subject,
36. The polynucleotide, vector or cell for use according to any one of paras 32-35, wherein the formation of geographic atrophy is prevented or reduced, and/or the amount of geographic atrophy is reduced.

37. The polynucleotide, vector or cell for use according to any one of paras 32-36, wherein the progression of geographic atrophy is slowed.
38. The polynucleotide, vector or cell for use according to any one of paras 32-37, 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.
39. The polynucleotide, vector or cell for use according to any one of paras 32-38, 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.
40. The polynucleotide, vector or cell for use according to any one of paras 32-39, wherein the polynucleotide, vector or cell is administered intraocularly.
41. The polynucleotide, vector or cell for use according to any one of paras 32-40, wherein the polynucleotide, vector or cell is administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection.
42. The polynucleotide, vector or cell for use according to any one of paras 32-41, wherein the polynucleotide, vector or cell is administered to the eye of a subject by subretinal injection.
43. A method of treating or preventing a complement-mediated disorder of the eye comprising administering the isolated polynucleotide, vector or cell of any one of paras 8-29 to a subject in need thereof.
44. A method of providing (i) an anti-VEGF entity; and (ii) a negative complement regulator to a subject, comprising delivering the isolated polynucleotide, vector or cell of any one of paras 8-29 to the eye of the subject.
45. An anti-VEGF entity for use in treating or preventing a disease, wherein the anti-VEGF entity is used in combination with a negative complement regulator.
46. A negative complement regulator for use in treating or preventing a disease, wherein the negative complement regulator is used in combination with an anti-VEGF entity.

47. An anti-VEGF entity for use according to para 45 or a negative complement regulator for use according to para 46, wherein the disease is a disease as recited in any of paras 2 to 6.
48. An anti-VEGF entity for use according to para 45 or 47 wherein the anti-VEGF entity is an entity as defined in any of paras 9 to 11.
49. A negative complement regulator for use according to para 46 or 47 wherein the negative complement regulator is an entity as defined in para 12 or 13.

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.

- 10 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.

25 **EXAMPLES**

EXAMPLE 1 - Codon optimisation of aflibercept sequence

The aflibercept protein sequence was first obtained from www.drugbank.ca (see SEQ ID NO: 1).

The protein sequence from 'Drugbank' was back translated into DNA sequence, which was then aligned with human VEGF Receptor and human IgG protein sequence and denoted as the 'wildtype' Aflibercept sequence. The wildtype aflibercept DNA sequence was derived and

subcloned into GT005 backbone denoted as RC288. A native FHL-1 signal peptide was added upstream of the wildtype aflibercept sequence to allow secretion from cells. Five online tools were used to generate “basic” sequences (GeneArt, Genscript, Genewiz, IDT & JCat). Each of the basic sequences were then manually optimised to remove cryptic splice sites, miRNA binding sites and tandem duplicate codons. These optimised sequences were designated as “manual”. Each codon optimised sequence was synthesised and cloned into GT005 backbone by Genewiz (Table 1 and Figure 1).

Table 1: Table enlisting variations of codon optimised Aflibercept constructs and reference links for online codon optimisation tools

Variant Number #	Plasmid Code	Codon Optimisation Tools	Reference Links
Wild type	RC288	Original “wild type”	Aflibercept sequence derived from drugbank.ca
1	RC290	GeneArt Basic	https://www.thermofisher.com/uk/en/home/life-science/cloning/gene-synthesis/geneart-gene-synthesis/geneoptimizer.html
2	RC291	GeneArt Manual	
3	RC292	Genscript Basic	https://www.genscript.com/
4	RC293	Genscript Manual	
5	RC294	GeneWiz Basic	https://www.genewiz.com/en-GB/
6	RC295	Genewiz Manual	
7	RC296	IDT Basic	https://eu.idtdna.com/pages/tools/codon-optimization-tool?returnurl=%2FCodonOpt
8	RC297	IDT Manual	
9	RC298	JCat Basic	http://www.jcat.de/
10	RC299	JCat Manual	

10

In general, the monocistronic Aflibercept vector is composed of:

- Inverted terminal repeats (ITR)
- CMV early enhancer/chicken β -actin (CAG) promoter
- Native or codon optimised FHL1 secretion signal peptide
- Aflibercept is composed of sequences from the second binding domain of vascular endothelial growth factor receptor VEGFR-1 and the third binding domain of VEGFR-2 fused to the sequence of a FC fragment of a human IgG (WT = wild type; coAflibercept = codon optimised Aflibercept sequence)
- Woodchuck hepatitis virus post-transcriptional regulatory element (WPRE)
- Bovine growth hormone poly-adenylation signal (bGHpA)

15

20

EXAMPLE 2 - Comparison of vectorised Aflibercept expression *in vitro*

All codon optimised Aflibercept vectors were packaged into AAV2 using triple transfection method. All vectors were then titred by qPCR and transduced into HEK293 at MOI 1E4. The level of Aflibercept expression was measured in culture supernatants 72 hr post-transduction by ELISA. The experiment was repeated twice. As shown in Figure 2, all codon optimised
5 Aflibercept vectors showed varying levels of expression.

In particular, RC298 (Jcat Basic) and RC299 (Jcat Manual) showed the highest level of Aflibercept expression as compared to control RC288 vector containing the non-codon optimised wild type sequence.

Western blot analysis was also carried out to confirm the integrity of the expressed Aflibercept
10 protein from the AAV2 vectors. All vectors demonstrated Aflibercept protein expression at the expected molecular weight of 115kDa (under non-reducing conditions), which was comparable to recombinant Aflibercept protein (Figure 3A and B). Densitometric analysis showed that RC298 expressed the highest level of Aflibercept (Figure 3C), consistent with the ELISA data as per Figure 2. In particular, RC299 showed an aberrant protein band pattern,
15 and was therefore excluded from selection.

In conclusion, RC298 was selected as the most optimal codon optimised Aflibercept sequence to take forward into the bicistronic vector.

EXAMPLE 3 – Construction of bicistronic vectors co-expressing negative complement regulator and Aflibercept

20 Bicistronic vectors were generated composed of a (CAG or CMV) promoter driving the expression of either codon optimised CFI or codon optimised FHL1 linked to codon optimised Aflibercept (derived from RC298) linked by a furin F2A linker. The position of the negative complement regulator and Aflibercept was interchanged between different configurations as shown in Figure 4 and Table 2.

25 In general, the bicistronic Aflibercept vectors are composed of:

- Inverted terminal repeats (ITR)
- Cytomegalovirus (CMV) promoter or CMV early enhancer/chicken β -actin (CAG) promoter
- Codon optimised complement factor I (CFI) or codon optimised FHL1
- 30 • Furin F2A cleavage peptide
- Aflibercept - composed of sequences from the second binding domain of vascular endothelial growth factor receptor VEGFR-1 and the third binding domain of VEGFR-2 fused to the sequence of a FC fragment of a human IgG (WT = wild type; coAflibercept = codon optimised Aflibercept sequence)

- Woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) or shortened version of WPRE containing only minimal gamma and alpha elements (WPRE3)
- Bovine growth hormone poly-adenylation signal (bGHpA)

5 Table 2: Table enlisting bicistronic vectors and corresponding configurations

Plasmid Code	Negative Complement Regulator	Anti-VEGF	Description of Packaged genome	SEQ ID NO
RC313	CFI (codon optimised)	Codon optimised Aflibercept sequence from RC298	ITR-CMV-coCFI-F2A-coAflibercept-WPRE3-bGHpA-ITR	45
RC289	CFI (codon optimised)	Codon optimised Aflibercept sequence from RC298	ITR-CMV-coAflibercept-F2A-coCFI-WPRE3-bGHpA-ITR	46
RC322	CFI (codon optimised)	Codon optimised Aflibercept sequence from RC298	ITR-CMV-coCFI-F2A-coAflibercept-bGHpA-ITR	47
RC323	CFI (codon optimised)	Codon optimised Aflibercept sequence from RC298	ITR-CMV-coAflibercept-F2A-coCFI-bGHpA-ITR	48
RC304	FHL1 (codon optimised)	Codon optimised Aflibercept sequence from RC298	ITR-CMV-coFHL1-F2A-coAflibercept-WPRE3-bGHpA-ITR	49
RC312	FHL1 (codon optimised)	Codon optimised Aflibercept sequence from RC298	ITR-CMV-coAflibercept-F2A-coFHL1-WPRE3-bGHpA-ITR	50
RC318	FHL1 (codon optimised)	Codon optimised Aflibercept sequence from RC298	ITR-CAG-coFHL1-F2A-coAflibercept-WPRE3-bGHpA-ITR	51
RC319	FHL1 (codon optimised)	Codon optimised Aflibercept sequence from RC298	ITR-CAG-coAflibercept-F2A-coFHL1-WPRE3-bGHpA-ITR	52

RC324	FHL1 (codon optimised)	Codon optimised Aflibercept sequence from RC298	ITR-CAG-coFHL1-F2A-coAflibercept-bGHpA-ITR	53
RC325	FHL1 (codon optimised)	Codon optimised Aflibercept sequence from RC298	ITR-CAG-coAflibercept-F2A-coFHL1-bGHpA-ITR	54

The codon optimised CFI sequence is SEQ ID NO: 35.

The codon optimised FHL1 sequence is SEQ ID NO: 41.

The codon optimised aflibercept sequence is SEQ ID NO: 11.

5 The 5' ITR is SEQ ID NO: 55.

The CMV promoter is SEQ ID NO: 59

The CAG promoter is SEQ ID NO: 60.

The WPRE sequence is SEQ ID NO: 63.

The WPRE3 sequence is SEQ ID NO: 64.

10 The bGHpA sequence is SEQ ID NO: 61.

The 3' ITR is SEQ ID NO: 57.

EXAMPLE 4 – Functional characterisation of bicistronic vectors co-expressing negative complement regulator and Aflibercept

15 Comparison of bicistronic vector expression was performed by *in vitro* transduction to compare expression of Aflibercept, CFI and FHL1 from bicistronic vectors listed in Figure 4 against monocistronic counterparts.

Functional analysis of Aflibercept from the bicistronic vectors was performed to determine anti-VEGF binding and anti-proliferation capabilities.

20 All bicistronic vectors were either packaged into AAV8 or AAV2 using triple transfection method. *In vitro* transduction was carried out in HEK293 cells at different MOIs to compare the expression of respective transgenes from bicistronic vectors as listed in Table 2. ELISA was used to quantify the expression levels of Aflibercept, CFI and FHL1 from the respective bicistronic vectors. In parallel, expression was compared to monocistronic counterparts. AAV8

and AAV2 bicistronic vectors (RC289 and RC313) showed measurable levels of CFI and Aflibercept expression following transduction, and expression levels from RC289 packaged as AAV8 and AAV2 vectors were comparable to monocistronic counterparts (Figures 7A and B). In contrast, RC313 showed lower levels of expression compared to RC289 and
5 corresponding monocistronic vectors. Similarly, AAV8 and AAV2 bicistronic vectors (RC304 and RC312) showed quantifiable levels of FHL-1 and Aflibercept expression and the levels of expression were comparable to that of corresponding monocistronic vectors (RC146 – monocistronic FHL1, RC298 – monocistronic Aflibercept) (Figures 7C and D).

In a further experiment, bicistronic vectors RC313, RC289, RC304 and RC312 were packaged
10 into AAV8 using triple transfection method. Purified vectors were transduced in HEK293 at 3 MOIs, in triplicate. Supernatants were taken and quantified by Aflibercept, CFI & FHL-1 ELISAs. For the CFI-Aflibercept bicistronic vectors (RC313 and RC289), Aflibercept expression was similar between the two vectors, and RC289 showed higher CFI expression than RC313 (Figure 7E). For the FHL-1-Aflibercept bicistronic vectors, Aflibercept expression
15 was again similar between the vectors, and RC304 showed higher FHL-1 expression at MOI 1E4 as compared to RC312 (Figure 7F). Using the same cell culture supernatant samples from expression studies, western blot analysis was carried out to assess protein expression from all bicistronic vectors. Under non-reducing conditions all cell culture supernatants showed CFI, FHL-1 and Aflibercept protein expression and all bicistronic vectors expressed the expected
20 protein size similar to that of the recombinant protein controls (see Figure 8).

These results indicate that all bicistronic vectors tested enabled dual transgene expression, and some candidates showed expression levels similar to that of monocistronic vectors. All bicistronic vectors expressed proteins of the correct size.

VEGF Binding Assay

25 To determine binding affinity of Aflibercept expressed from the bicistronic vectors for VEGFA, an equilibrium binding assay was performed. HEK293 cells were transduced with monocistronic vectors expressing Aflibercept (RC298), and bicistronic vectors co-expressing CFI or FHL1 and Aflibercept. 72 hours post-transduction, secreted Aflibercept in the culture supernatant was extracted and quantified by ELISA. Binding affinities of secreted Aflibercept
30 derived from vectors and recombinant Aflibercept (Eylea®) was measured by using a specific and sensitive ELISA for detecting free (unbound) human VEGFA in mixtures of the Aflibercept (across a range of concentration) with human VEGFA incubated over a fixed period of time. As shown in Figure 9, the binding equilibriums (kD) of Aflibercepts [IC50] for VEGFA derived from both monocistronic and bicistronic vectors were found to be similar to that of Eylea®.

These data demonstrate that the designed bicistronic vectors were able to express Aflibercept that functions at a similar capacity to that of Eylea®.

VEGF-induced Proliferation Assay

To determine whether AAV vector derived Aflibercept effectively binds VEGFA and effectively blocks the ability of VEGF to induce cell proliferation, human umbilical vascular endothelial (HUVEC) cells were challenged with a set concentration of human VEGFA and varying concentrations of Aflibercept (derived from cultured supernatant following AAV transduction of HEK293 cells as above) or Eylea® (positive control). 72 hr post-treatment, inhibition of VEGF-induced proliferation of HUVEC cells was measured by the addition of [3-(4,5 dimethylthiazol-2-yl)-5-(3-carboxymethoxypheyl)-2-(4-sulfophenyl)-2H-tetrazolium, and spectrophotometric analysis at 450/570nm. As shown in Figure 10, Aflibercept derived from monocistronic and bicistronic vectors blocked VEGF-induced proliferation in a 5-day growth assays in these cells, and the anti-proliferative properties observed were similar to that of Eylea®.

These data demonstrate that the designed bicistronic vectors are able to express Aflibercept that functions at a similar capacity to that of Eylea®.

C3b Cleavage Assays

To analyse the functional activity of CFI or FHL1 secreted from transduced cells, conditioned supernatant from HEK293 cells transduced with: AAV2 expressing CFI (GT005); AAV2 expressing FHL1 (RC146); AAV2 co-expressing CFI and Aflibercept (RC289 or RC313), or AAV2 co-expressing FHL1 and Aflibercept (RC312 or RC304) was tested in a C3b cleavage assay (Figure 11). The principle of this assay is based on the ability of CFI, in the presence of FHL1, to cleave C3b to iC3b. iC3b ELISA quantifies the amount of C3b breakdown product, iC3b.

C3b cleavage assays were conducted by incubating 1ug C3b with recombinant FHL1 (GTP Tech) and supernatant from cells transduced with CFI expressing vectors (GT005, RC289 and RC313) at a 4:1 molar ratio of FHL1: CFI. The reaction volume was made up to 20ul with Opti-MEM™ reduced serum media and reactions were incubated at 37 degrees for 20 minutes. Supernatant from cells transduced with FHL1 vectors (RC146, RC312 or RC304) was incubated with recombinant CFI (Complement Technology, Inc, A138), 1ug C3b (Complement Technology, Inc, A113) and Opti-MEM™ serum free media at a 4:1 molar ratio of FHL1: CFI at 37°C for 20 minutes in a total volume of 20ul.

Resultant samples were diluted 1:100 and 1:400 and loaded onto an iC3b ELISA using coating antibody: mouse anti-human activated C3 (Hycult, HM2168), detection antibody: rat anti-C3dg (Hycult, HM2199), and secondary antibody: mouse anti-rat (Southern Biotech, 3061-05). iC3b concentrations (ng/mL) were calculated according to a standard curve generated using iC3b purified human protein (Complement Technology, Inc).

C3b incubated with recombinant CFI and FHL1 served as a positive control (Figure 11, left hand bars). The C3b only negative control demonstrated that C3b breakdown requires the presence of CFI and FHL1 as C3b is stable in the absence of additional components. C3b incubated with conditioned supernatant from untransduced cells (UTD) also served as a negative control. iC3b levels from bicistronic vectors were compared to monocistronic counterparts. Results of iC3b ELISA demonstrated that CFI and FHL1 expressed from all bicistronic vectors is functional and can facilitate breakdown of C3b to iC3b (see Figure 11).

In vivo studies

In vivo bicistronic expression and efficacy studies are performed to determine expression of negative complement regulator and Aflibercept from bicistronic vectors in rodents. Studies are also performed to demonstrate efficacy of bicistronic vectors in a laser CNV mouse model.

Example 5 - Administration of monocistronic vectors expressing CFI or FHL1 in a mouse model of CNV

AAV vectors are first delivered subretinally into the mouse eye and left for 4 weeks. At Day 0, all pre-injected eyes are subjected to laser induced CNV lesions by laser burning of the Bruch's membrane. Aflibercept (Eylea®) is also delivered via intravitreal injection into CNV lesion eyes for the positive control group. Optical coherence tomography and fluorescein angiography are used to image the retinal structure and CNV lesion areas respectively at Day 0 and Day 4. At Day 7 post-laser burn, all mice are culled and CNV areas (determined by isolectin staining), CNV leakage area and CNV score (graded) are measured as primary and secondary endpoints (see Figure 6).

Example 6 – In vivo expression studies

Monocistronic Expression data

AAV8 (monocistronic) vectors expressing anti-VEGF (Aflibercept) were administered subretinally into the right eye of nine-week-old C57BL/6JRj male mice as per groups below. Successful subretinal administrations were verified using spectral-domain optical coherence tomography (SD-OCT)

- Group 1: AAV8 RC298, dose 5e7 vg/eye (n = 9);
- Group 2: AAV8 RC298, dose 2e8 vg/eye (n = 10);
- Group 3: AAV8 RC298, dose 5e8 vg/eye (n = 10);
- Group 4: AAV8 RC298, dose 2e9 vg/eye (n = 10);
- 5 Group 5: AAV8 RC298, dose 5e9 vg/eye (n= 10);
- Group 6: AAV8 RC298, dose 1e10 vg/eye (n = 9).

The contralateral eye of each animal served as uninjected control. Five weeks post-injection, all animals were sacrificed. Both eyes, injected and contralateral uninjected, were dissected and ocular fluids were collected in 40 µl of PBS (containing protease inhibitors) in screw capped tubes and snap frozen until protein analysis. Posterior eyecups were placed into a fresh tube containing 50 µl of RNAlater solution and were broken down further with dissecting scissors. The dissected eyecups were then snap frozen in liquid nitrogen for 30 sec, and 50 uL RLT buffer containing β-mercaptoethanol was added before subjecting to further homogenisation using a homogeniser for 2 min. 200 uL RLT buffer with β-mercaptoethanol was added to the eyecup homogenates and mixed by pipetting up and down. All eyecup homogenates were stored for at least 24 hr at -80°C before RNA extraction.

RNA extraction and quantitative RT-PCR

RNA was isolated from rat posterior eyecup tissues using RNeasy Mini kit as per manufacturer's protocol (Qiagen). Frozen eyecup homogenates were thawed on ice subjected to mechanical shredding by using a QIAshredder. Following shredding, 70% EtOH was added to the homogenate and the mixture was then transferred to a RNeasy column. The column was centrifuged and washed with RW1 buffer. The column membrane was then treated with DNaseI by incubation at room temperature for 15 mins before further washing with RW1 and RPE buffers. RNA was then eluted with RNase-free water in a final volume of 50 µL. RNA concentration of each sample was then measured using NeoDot nanospectrometer (Generon).

The reverse transcription was performed using the SuperScript III Reverse Transcriptase Kit (Invitrogen). Transgene-derived mRNA was quantified by using the following primers targeting the bGHpA sequence for murine samples: bGHpA FR 5'-CATCGCATTGTCTGAGTAGGT-3' and bGHpA Rv 5'-AGCATGCCTGCTATTGTCTT-3'. All qRT-PCR was conducted using an CFX96 Touch Real-Time PCR Detection System (BioRad) with SYBR Green chemistry. As a standard, linearised transgene plasmid diluted to known concentrations was used. The SYBR Green applications were performed as follows: initial denaturation at 95°C for 3 min followed by 40 cycles of 10 sec at 95°C and 30 sec at 56°C.

Figure 12 shows the Aflibercept mRNA expression for Groups 1 to 6.

Quantitation of Aflibercept expression

Unbound aflibercept in ocular fluids was measured using a quantitative sandwich type ELISA as per manufacturer's protocol (ImmunoGuide). In brief, diluted Aflibercept standards and ocular fluid samples were incubated in the microtiter plate coated with recombinant human vascular endothelial growth factor-A (rhVEGF-A). After incubation, the wells were washed and horseradish peroxidase (HRP) conjugated anti-human IgG monoclonal antibody was added to bind to the Fc portion of Aflibercept pre-captured by the rhVEGF-A on the surface of the wells. Following incubation, the wells were washed, and the bound enzymatic activity was detected by the addition of chromogen-substrate. The colour developed was proportional to the amount of Aflibercept in the sample or standard, and the optical density (OD) was measured with a photometer at 450 nm (reference at OD620 nm was optional).

Figure 13 shows the Aflibercept protein expression for Groups 1 to 6.

Bicistronic Expression data

Bicistronic AAV vectors co-expressing antiVEGF (Aflibercept) and complement regulators (hCFI or FHL-1) were administered subretinally into the right eye (OD) of 10-week-old C57BL/6 male mice. Successful subretinal administrations were verified using spectral-domain optical coherence tomography (SD-OCT). The contralateral eye of each animal served as an uninjected control. After four weeks the mice were sacrificed, and ocular fluids and posterior eye cups were collected.

The study consisted of eight treatment groups:

- Group 1: AAV8-RC313, 5×10^7 vg/eye (n= 10 mice);
- Group 2: AAV8-RC313, 5×10^8 vg/eye (n= 10);
- Group 3: AAV8-RC289, 5×10^7 vg/eye (n= 10);
- Group 4: AAV8-RC289, 5×10^8 vg/eye (n= 10);
- Group 5: AAV8-RC304, 5×10^7 vg/eye (n= 9);
- Group 6: AAV8-RC304, 5×10^8 vg/eye (n= 10);
- Group 7: AAV8-RC312, 5×10^7 vg/eye (n= 9);
- Group 8: AAV8-RC312, 5×10^8 vg/eye (n= 10).

RNA extraction and quantitative RT-PCR

Posterior eyecups were placed into a fresh tube containing 50 μ l of RNAlater solution and were broken down further with dissecting scissors. The dissected eyecups were then snap

frozen in liquid nitrogen for 30 sec, and 50 μ L RLT buffer containing β -mercaptoethanol was added before subjecting to further homogenisation using a homogeniser for 2 min. 200 μ L RLT buffer with β -mercaptoethanol was added to the eyecup homogenates and mixed by pipetting up and down. All eyecup homogenates were stored for at least 24 hr at -80°C before
 5 RNA extraction.

- RNA was isolated from rat posterior eyecup tissues using RNeasy Mini kit as per manufacturer's protocol (Qiagen). Frozen eyecup homogenates were thawed on ice subjected to mechanical shredding by using a QIAshredder. Following shredding, 70% EtOH was added to the homogenate and the mixture was then transferred to a RNeasy column. The
 10 column was centrifuged and washed with RW1 buffer. The column membrane was then treated with DNaseI by incubation at room temperature for 15 mins before further washing with RW1 and RPE buffers. RNA was then eluted with RNase-free water in a final volume of 50 μ L. RNA concentration of each sample was then measured using NeoDot nanospectrometer (Generon).
- 15 The reverse transcription was performed using the SuperScript III Reverse Transcriptase Kit (Invitrogen). Transgene-derived mRNA was quantified by using the following primers targeting the bGHpA sequence for murine samples: bGHpA FR 5'-CATCGCATTGTCTGAGTAGGT-3' and bGHpA Rv 5'-AGCATGCCTGCTATTGTCTT-3'. All qRT-PCR was conducted using an CFX96 Touch Real-Time PCR Detection System (BioRad) with SYBR Green chemistry. As a
 20 standard, linearised transgene plasmid diluted to known concentrations was used. The SYBR Green applications were performed as follow: initial denaturation at 95°C for 3 min followed by 40 cycles of 10 sec at 95°C and 30 sec at 56°C .

Figure 14 shows the Aflibercept mRNA expression from the bicistronic vectors

Quantitation of Aflibercept and complement expression

25 *Aflibercept*

- Unbound aflibercept in ocular fluids was measured using a quantitative sandwich type ELISA as per manufacturer's protocol (ImmunoGuide). In brief, diluted Aflibercept standards and ocular fluid samples were incubated in the microtiter plate coated with recombinant human vascular endothelial growth factor-A (rhVEGF-A). After incubation, the wells were washed and
 30 horseradish peroxidase (HRP) conjugated anti-human IgG monoclonal antibody was added to bind to the Fc portion of Aflibercept pre-captured by the rhVEGF-A on the surface of the wells. Following incubation, the wells were washed, and the bound enzymatic activity was detected by the addition of chromogen-substrate. The colour developed was proportional to

the amount of Aflibercept in the sample or standard, and the optical density (OD) was measured with a photometer at 450 nm (reference at OD620 nm was optional).

CFI protein expression in ocular fluids

Human CFI in ocular fluids was quantified using MSD's electrochemiluminescence detection technology. An MSD Gold 96-well small spot streptavidin Sector plate was coated with biotinylated anti-CFI antibody shaking (850RPM) for 1 hr at room temperature. CFI Standards (Complement Technology) and samples were diluted accordingly. The plate was washed with PBS-Tween 0.05% and 25 uL of standards, controls and samples were added to the plate. The plate was sealed and incubated shaking (850RPM) at room temperature for 1 hour. The plate was then washed as previous and 25 uL of anti FI (anti-FI, A231) detection antibody was added to the wells and incubated shaking (850RPM) at room temperature for 1 hour. Following incubation, the plate was washed as previous and 150ul of 2X Read Buffer T was added to each well before reading on the MSD plate reader.

FHL-1 protein expression in ocular fluids

FHL-1 in ocular fluids was quantified using MSD's electrochemiluminescence detection technology. An MSD Gold 96-well streptavidin Sector plate was coated with biotinylated anti-FHL-1 antibody shaking (850RPM) for 1 hr at room temperature. FHL-1 standards and samples were diluted accordingly. The plate was washed with PBS-Tween 0.05% and 25 uL of standards, controls and samples were added to the plate. The plate was sealed and incubated shaking (850RPM) at room temperature for 1 hour. The plate was then washed as previous and 25 uL of anti FH detection antibody (anti-FH, MA170057, Invitrogen) was added to the wells and incubated shaking (850RPM) at room temperature for 1 hour. Following incubation, the plate was washed as previous and 150ul of 2X Read Buffer T was added to each well before reading on the MSD plate reader.

Figure 15 shows the Aflibercept, CFI and FHL1 protein expression from the bicistronic vectors.

Example 7 – In vivo efficacy in murine laser-induced choroidal neovascularisation model

Five groups of mice received unilateral (right eye) subretinal injections of different AAV vectors. Successful subretinal treatment administration was confirmed immediately after AAV administration using spectral-domain optical coherence tomography (SD-OCT). Four weeks after administration of the vectors, CNV was induced by laser photocoagulation. In brief, anaesthetized animals received a drop of 0.5% tropicamide (Oftan Tropicamid, Santen Oy) to dilate the pupils. A drop of Viscotears (Dr. Gerhard Mann Chem. -Pharm., Germany) was

applied on the eye and a coverslip was used to appanate the cornea. Three laser lesions were executed unilaterally on the right eye around the optic nerve head using a 532 nm diode laser (spot size: 100 μ m; power: 130 mW; time: 120 ms. Oculight TX. Iridex Corp., USA). The successful production of CNV lesions was confirmed by using SD-OCT and fluorescein angiography (FA). Immediately after CNV induction, an additional group of mice received intravitreal administration of aflibercept. Mice were imaged again at days four and seven post laser

Lasere eyes were enucleated and dissected. Choroidal flat mounts were prepared and stained with fluorescein-labelled Griffonia Simplicifolia Lectin I (GSLI) isolectin B4 (FL-1201, Vector Laboratories, USA) to evaluate the neovascularization. Briefly, the flat mounts were washed with Tris-buffered saline (TBS) and blocked with 10 % normal goat serum (NGS), 0.5 % Triton X-100 in TBS pH 7.4 (TBST) for 1 hour in room temperature (RT). Samples were washed with TBS and incubated with fluorescein-labelled Isolectin GS-IB4 (1:200, Vector Laboratories) overnight at +4°C in 1 % NGS diluted in 0.1 % TBST. Thereafter the samples were washed 3 x 10 min with 1 % NGS diluted in 0.1 % TBST, counterstained with DAPI and mounted with Fluoroshield™ mounting medium (Sigma-Aldrich) on microscopic slides. Choroidal samples were imaged using a DMI8 THUNDER 3D microscope (Leica Microsystems, Germany). The stained areas were outlined and the stained area was measured using the image processing software FIJI (Schindelin et al. 2012).

Group 1: RC268, null, 5E8 on Day -28 and CNV induction;

Group 2: RC289, 5E7 on Day -28 and CNV induction;

Group 3: RC289, 5E8 on Day -28 and CNV induction;

Group 4: RC304, 5E7 on Day -28 and CNV induction;

Group 5: RC304, 5E8 on Day -28 and CNV induction;

Group 6: CNV induction and aflibercept (80 μ g/eye) treatment on day 0.

Quantitation of CNV leakage area and CNV lesion size

Fluorescein Angiography (FA): Vascular leakage at the choroid level was examined using a Heidelberg Spectralis HRA system (Heidelberg Engineering, Germany). Briefly, a drop of 0.5% tropicamide (Oftan Tropicamid. Santen Oy) was administered on the cornea of the anaesthetized mouse, in order to dilate the pupils, and the mouse was placed onto the mouse holder. After aligning the optic nerve head at the retina level, with the use of the infrared reflectance camera, a solution of 2.5 % sodium fluorescein (Sigma-Aldrich,

Finland) was administered as a s.c. injection (30 μ l/10 g). Consecutive fluorescent images (Sensitivity: 45; ART Mean: 5 frames) were taken every 60 sec from the retinal and choroidal focus levels for a period of 5 min after the fluorescein administration.

Spectral Domain Optical Coherence Tomography (SD-OCT): SD-OCT was performed to verify the subretinal administration, prior and after the CNV induction, and at days 4 and 7 after CNV induction. Immediately after the FA imaging the mouse was examined using the SD-OCT system Envisu R2200 (Bioptigen Inc./Leica Microsystems, USA). The scanned area covers a 1.4 x 1.4 mm² of the retina centered around the optic nerve. Each scan is composed of 100 B Scans each one composed of 1000 A Scans.

- 5
- 10 CNV leakage area as assessed by FA fundus imaging and subsequent AI image analysis. FA scans were analyzed by a proprietary algorithm, which uses a combination of convolutional neural network (CNN) designed for semantic segmentation and traditional computer vision algorithms. The neural network was trained to recognize and quantify CNV lesions using transfer learning approach. The results from the model were reviewed and adjusted if necessary by a scientist blinded to the treatments
- 15

The lasered areas were qualitatively graded as being leaky or non-leaky from FA scans and verified from SD-OCT scans.

Data Analysis

Quantitative data was graphed, analyzed and presented as mean $\pm$ standard deviation (SD).

- 20 Statistical analyses were performed using the GraphPad Prism software (v8.0.1 GraphPad Software, USA). Differences were considered statistically significant at the $p < 0.05$ level.

The results are shown in Figure 16.

- 25 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.
- 30

CLAIMS

1. An isolated polynucleotide comprising nucleotide sequences encoding (i) an anti-VEGF entity and (ii) a negative complement regulator.
- 5 2. The isolated polynucleotide according to claim 1 wherein the anti-VEGF entity is selected from an Ig fusion protein, an antibody, a polypeptide, a peptide, a non-antibody scaffold, an antisense oligonucleotide, siRNA, shRNA, CRISPR-guide strand and an aptamer.
3. The isolated polynucleotide according to claim 2 wherein the anti-VEGF entity is selected from aflibercept, ranibizumab, bevacizumab, brolucizumab and pegaptanib.
- 10 4. The isolated polynucleotide according to claim 3 wherein the anti-VEGF entity is aflibercept, for example wherein the aflibercept is encoded by a polynucleotide sequence comprising any one of SEQ ID NO: 3 to 11.
5. The isolated polynucleotide according to any preceding claim wherein the negative complement regulator is selected from Complement Factor I (CFI), Complement Factor H Like Protein 1 (FHL1), Complement Factor H (CFH), Complement receptor type 1 (CR1), Membrane Cofactor Protein (MCP), Complement decay-accelerating factor (DAF), MAC-inhibitory protein (MAC-IP), C1-inhibitor, anaphylatoxins inhibitor, C4b binding protein (C4BP), clusterin, vitronectin or a variant or fragment thereof.
- 15 6. The isolated polynucleotide according to claim 5 wherein the negative complement regulator is selected from CFI and FHL1 or a variant or fragment thereof.
7. The isolated polynucleotide according to any of claims 3 to 6, wherein the nucleotide sequence encoding aflibercept has at least 75% sequence identity to SEQ ID NO: 11.
8. The isolated polynucleotide according to claim 6 or 7, wherein the nucleotide sequence encoding CFI has at least 75% sequence identity to SEQ ID NO: 35 or 36 or the nucleotide sequence encoding FHL-1 has at least 75% sequence identity to SEQ ID NO: 41.
- 25 9. The isolated polynucleotide according to any of claims 3 to 8, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 45 to 54, or a nucleotide sequence that has at least 75% sequence identity thereto.
- 30 10. The isolated polynucleotide according to any of claims 1 to 9, wherein the polynucleotide is less than or equal to 4.7 kb.

11. A vector comprising the isolated polynucleotide of any one of claims 1 to 10.
12. The vector of claim 11, wherein the vector is an adeno-associated viral (AAV) vector, optionally in the form of a viral vector particle, preferably an AAV vector particle comprising an AAV2 genome and AAV2 or AAV8 capsid proteins.
- 5 13. A pharmaceutical composition comprising the isolated polynucleotide or vector of any one of claims 1 to 12 in combination with a pharmaceutically acceptable carrier, diluent or excipient.
14. The isolated polynucleotide, vector or pharmaceutical composition of any one of claims 1 to 13 for use in therapy.
- 10 15. The isolated polynucleotide, vector or pharmaceutical composition of any one of claims 1 to 14 for use in treating or preventing a complement-mediated disorder of the eye, for example age-related macular degeneration (AMD), diabetic retinopathy, glaucoma, Stargardt's disease, central serous chorioretinopathy, retinitis pigmentosa, polypoidal choroidal vasculopathy, diabetic macular edema, branch retinal vein occlusion or
- 15 uveitis, preferably AMD.
16. The isolated polynucleotide, vector or pharmaceutical composition for use according to claim 15, wherein the AMD is wet AMD and/or dry AMD.
17. The isolated polynucleotide, vector or pharmaceutical composition for use according to claim 15, wherein i) the AMD is wet AMD and the use further prevents and/or treats
- 20 onset of dry AMD in a subject, or ii) the AMD is dry AMD and the use further prevents and/or treats onset of wet AMD in a subject, or iii) the use prevents and/or treats dry AMD and wet AMD simultaneously.
18. The isolated polynucleotide, vector or pharmaceutical composition for use according to claim 17, wherein the AMD is wet AMD and the use further prevents and/or treats
- 25 onset of dry AMD in a subject.
19. The isolated polynucleotide, vector or pharmaceutical composition for use according to any of claims 15 to 18, wherein:
- (i) the formation of geographic atrophy is prevented or reduced, and/or the amount of geographic atrophy is reduced or wherein the progression of geographic atrophy is
- 30 slowed;

- (ii) 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; or
- (iii) administration of the isolated polynucleotide, vector or pharmaceutical composition 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.
20. The isolated polynucleotide, vector or pharmaceutical composition for use according to any of claims 15 to 19, wherein i) neovascularization is prevented or reduced, ii) vascular leakage is prevented or reduced and/or iii) retina edema is prevented or reduced.
21. The isolated polynucleotide, vector or pharmaceutical composition for use according to any one of claims 15 to 20, wherein the polynucleotide, vector, cell or pharmaceutical composition is administered intraocularly, for example by subretinal, direct retinal, suprachoroidal or intravitreal injection.
22. A product comprising (i) an anti-VEGF entity; and (ii) a negative complement regulator, or nucleotide sequences encoding therefor, as a combined preparation for i) simultaneous, separate or sequential use in treating wet AMD and wherein the use further prevents and/or treats onset of dry AMD in a subject; or ii) simultaneous, separate or sequential use in treating dry AMD and wherein the use further prevents and/or treats onset of wet AMD in a subject, or iii) simultaneous, separate or sequential use for simultaneously treating and/or preventing dry AMD and wet AMD.
23. The product for use according to claim 22 for simultaneous, separate or sequential use in treating wet AMD and wherein the use further prevents and/or treats onset of dry AMD in a subject.
24. The product for use according to claim 22 or 23 wherein the anti-VEGF entity is selected from an Ig fusion protein, an antibody, a polypeptide, a peptide, a non-antibody scaffold, an antisense oligonucleotide, siRNA, shRNA, CRISPR-guide strand and an aptamer, preferably wherein the anti-VEGF entity is selected from aflibercept, ranibizumab, bevacizumab, brolucizumab and pegaptanib.
25. The product for use according to any of claims 22 to 24 wherein the negative complement regulator is selected from Complement Factor I (CFI), Complement

- Factor H Like Protein 1 (FHL1), Complement Factor H (CFH), Complement receptor type 1 (CR1), Membrane Cofactor Protein (MCP), Complement decay-accelerating factor (DAF), MAC-inhibitory protein (MAC-IP), C1-inhibitor, anaphylatoxins inhibitor, C4b binding protein (C4BP), clusterin, vitronectin or a variant or fragment thereof, preferably wherein the negative complement regulator is selected from CFI or FHL1 or a variant or fragment thereof.
26. A product comprising (i) an anti-VEGF entity; and (ii) Factor I, or nucleotide sequences encoding therefor, as a combined preparation for simultaneous, separate or sequential use as a medicament.
27. The product for use according to claim 26 for use in treating or preventing a complement-mediated ocular disorder, such as age-related macular degeneration (AMD), diabetic retinopathy, glaucoma, Stargardt's disease, central serous chorioretinopathy, retinitis pigmentosa, polypoidal choroidal vasculopathy, diabetic macular edema, branch retinal vein occlusion or uveitis, preferably AMD.
28. The product for use according to any of claims 22 to 27, wherein:
- (i) the formation of geographic atrophy is prevented or reduced, and/or the amount of geographic atrophy is reduced or wherein the progression of geographic atrophy is slowed;
 - (ii) 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; or
 - (iii) administration of the isolated polynucleotide, vector or pharmaceutical composition 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.
29. The product for use according to any of claims 22 to 28, wherein i) neovascularization is prevented or reduced, ii) vascular leakage is prevented or reduced and/or iii) retina edema is prevented or reduced.