A therapy for the treatment of geographic atrophy has been developed and is currently in a phase III clinical study (MAHALO study by Genentech/Roche). Lampalizumab is a humanised monoclonal inhibitory antibody to complement Factor D, administered by intravitreal injection to stop the rate of progression of geographic atrophy. As show in Figure 1, Factor D is part of the C3b feedback ('amplification') cycle. Factor D is present in very low serum concentrations and is an essential factor for the alternative pathway. Nevertheless, due to its small size (27 kDa), Factor D is rapidly cleared out by the kidneys and quickly re-synthesised. The therapy requires monthly intravitreal injections.

There is a need in the art for new approaches to treat AMD.

SUMMARY

The present inventors now provide an approach for modulating the complement system which is useful, for example, in the treatment of AMD. The inventors provide Factor I delivered by gene therapy with the aim of negatively regulating the complement C3b feedback cycle through targeting of the breakdown cycle (Figure 1). The resulting re-balancing of the feedback loop of the alternative pathway will promote C3b and iC3b breakdown and thus remove major disease factors in complement-mediated disorders, particularly disorders that have an underlying defect in alternative pathway regulation. Alternatively, Factor H may instead be used.

In one aspect, the present disclosure refers to an adeno-associated viral (AAV) vector comprising a nucleotide sequence encoding Factor I or a fragment or derivative thereof, wherein the Factor I, fragment, or derivative is capable of cleaving C3b into iC3b. In another aspect, the present disclosure refers to an isolated cell transfected with the AAV vector as disclosed herein. In another aspect, the present disclosure refers to a pharmaceutical composition comprising the AAV vector or the cell as disclosed herein in combination with a pharmaceutically acceptable carrier, diluent or excipient. In yet another aspect, there is provided an AAV vector, cell or pharmaceutical composition of the present disclosure for use in treating or preventing a complement-mediated disorder of the eye, wherein the disorder is age-related macular degeneration (AMD) or diabetic retinopathy. In yet another aspect, there is provided the use of the AAV vector, cell or pharmaceutical composition of the present disclosure in the manufacture of a medicament for treating or preventing a complement-mediated disorder of the eye, wherein the disorder is age-related macular degeneration (AMD) or diabetic retinopathy.

In one example, the disclosure provides an adeno-associated viral (AAV) vector comprising a nucleotide sequence encoding Factor I or a fragment or derivative thereof. In another

example, the disclosure provides an adeno-associated viral (AAV) vector comprising a nucleotide sequence encoding Factor H or a fragment or derivative thereof. In another example, the disclosure provides an adeno-associated viral (AAV) vector comprising a nucleotide sequence encoding an anti-Factor D antibody. In another example, the disclosure provides an adeno-associated viral (AAV) vector comprising a nucleotide sequence encoding an anti-complement component 5 (C5) antibody.

In one example, the nucleotide sequence encoding Factor I or fragment or derivative thereof comprises a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding an amino acid sequence that has at least 70% identity to SEQ ID NO: 1 or 9;

(b) a nucleotide sequence that has at least 70% identity to SEQ ID NO: 2 or 8; and

(c) the nucleotide sequence of SEQ ID NO: 2 or 8.

Preferably, the nucleotide sequence encoding Factor I or fragment or derivative thereof encodes a protein with the natural activity of Factor I (e.g. the protein represented by SEQ ID NO: 1 or 9). For example, the nucleotide sequence encoding Factor I or fragment or derivative thereof may encode a protein with the ability to process C3b and iC3b into inactive degradation products. Put another way, the Factor I or fragment or derivative thereof preferably retains C3b-inactivating and iC3b-degradation activity.

In a preferred example, the nucleotide sequence encoding Factor I or fragment or derivative thereof encodes a protein with C3b-inactivating and iC3b-degradation activity.

In one example, the nucleotide sequence encoding Factor H or fragment or derivative thereof comprises a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding an amino acid sequence that has at least 70% identity to SEQ ID NO: 3;

(b) a nucleotide sequence that has at least 70% identity to SEQ ID NO: 4; and

(c) the nucleotide sequence of SEQ ID NO: 4.

Preferably, the nucleotide sequence encoding Factor H or fragment or derivative thereof encodes a protein with the natural activity of Factor H (e.g. the protein represented by SEQ

ID NO: 3). For example, the nucleotide sequence encoding Factor H or a fragment or derivative thereof may encode a protein with the ability to act as a cofactor for the Factor I mediated cleavage of C3b and to increase the rate of dissociation of C3 convertase and C5 convertase.

In another example, the disclosure provides a cell transfected with the AAV vector of the disclosure.

In another example, the disclosure provides a pharmaceutical composition comprising the AAV vector of the disclosure or the cell of the disclosure in combination with a pharmaceutically acceptable carrier, diluent or excipient. In a preferred example, the pharmaceutical composition is for intraocular administration.

In one example, the AAV vector comprises a chicken beta-actin (CBA) promoter, for example operably linked to the nucleotide sequence encoding Factor I or H or fragment or derivative thereof. In one example, the AAV vector comprises a CAG promoter, for example operably linked to the nucleotide sequence encoding Factor I or H or fragment or derivative thereof. In one example, the AAV vector comprises a promoter with the nucleotide sequence of SEQ ID NO: 5, for example operably linked to the nucleotide sequence encoding Factor I or H or fragment or derivative thereof.

In one example, the AAV vector comprises a cytomegalovirus (CMV) enhancer element, for example operably linked to the nucleotide sequence encoding Factor I or H or fragment or derivative thereof.

In one example, the AAV vector comprises a Bovine Growth Hormone poly-A signal, for example operably linked to the nucleotide sequence encoding Factor I or H or fragment or derivative thereof, preferably a Bovine Growth Hormone poly-A signal having the nucleotide sequence of SEQ ID NO: 6.

In one example, the AAV vector comprises a woodchuck hepatitis post-transcriptional regulatory element (WPRE), for example operably linked to the nucleotide sequence encoding Factor I or H or fragment or derivative thereof, preferably a WPRE having the nucleotide sequence of SEQ ID NO: 7.

In a preferred example, the AAV vector of the disclosure is in the form of a viral particle.

In a preferred example, the AAV viral particle comprises an AAV2 genome and AAV2 capsid proteins. Preferably, the nucleotide sequence encoding Factor I or H or fragment or

derivative thereof is operably linked to a CAG promoter, preferably a promoter with the nucleotide sequence of SEQ ID NO: 5.

The AAV vector, cell or pharmaceutical composition of the disclosure may be used to treat or prevent an ocular disorder.

In one example, the disclosure provides the AAV vector, cell or pharmaceutical composition of the disclosure for use in treating or preventing a complement-mediated disorder of the eye.

In one example, the disorder is associated with over-activity of the complement C3b feedback cycle and/or under-activity of the C3b breakdown cycle (see Figure 1). In one example, the disorder is age-related macular degeneration (AMD) or diabetic retinopathy. In a preferred example, the disorder is AMD, preferably dry AMD.

In one example, the use is for treating or preventing a disorder in a subject:

(a) having lower than normal Factor I activity or concentration in the eye and/or serum, preferably having a concentration of, or activity equivalent to, 0-30 or 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 Factor I variant.

In one example, the use is for treating or preventing a disorder in a subject:

(a) having a normal level of 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 Factor I variant allele.

In another example, the disclosure provides the AAV vector, cell or pharmaceutical composition of the disclosure for use in treating or preventing age-related macular degeneration (AMD). The AMD may, for example, be dry AMD. In a preferred example, the AMD is dry AMD.

In another example, the disclosure provides the AAV vector, cell or pharmaceutical composition of the disclosure for use in treating or preventing diabetic retinopathy.

In one example, the formation of geographic atrophy is prevented or reduced. In another example, the amount of geographic atrophy is reduced.

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

In another example, the disclosure provides the AAV vector, cell or pharmaceutical composition of the disclosure 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 example, the disclosure provides the AAV vector, cell or pharmaceutical composition of the disclosure 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 example, the disclosure provides the AAV vector, cell or pharmaceutical composition of the disclosure 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 example, the disclosure provides the AAV vector, cell or pharmaceutical composition of the disclosure 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 example, the disclosure provides the AAV vector, cell or pharmaceutical composition of the disclosure 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 preferred example, the AAV vector, cell or pharmaceutical composition for use according to the disclosure is administered intraocularly. The inventors recognise that such local administration of the therapy provides a means practically to achieve the required levels of the complement factor for treating or preventing the complement-mediated disorder of the eye, for example AMD.

In one example, the AAV vector, cell or pharmaceutical composition of the disclosure is administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection.

In a particularly preferred example, the AAV vector, cell or pharmaceutical composition of the disclosure is administered to the eye of a subject by subretinal injection.

In one example, administration of the AAV vector, cell or pharmaceutical composition of the disclosure thereby increases the level of C3b-inactivating and iC3b-degradation activity in the subject, in particular in the eye, such as in the RPE, of the subject. In another example, administration of the AAV vector, cell or pharmaceutical composition of the disclosure thereby increases the level of C3b-inactivating and iC3b-degradation activity in the subject, in particular in the eye, such as in the RPE, of the subject to a level that exceeds a normal level in the eye.

In another example, the disclosure provides a method of treating or preventing a complement-mediated disorder of the eye comprising administering the AAV vector, cell or pharmaceutical composition of the disclosure to a subject in need thereof.

In another example, the disclosure provides a use of the AAV vector, cell or pharmaceutical composition of the present disclosure in the manufacture of a medicament for treating or preventing a complement-mediated disorder of the eye.

In one example, the disorder is associated with over-activity of the complement C3b feedback cycle and/or under-activity of the C3b breakdown cycle. In one example, the disorder is age-related macular degeneration (AMD) or diabetic retinopathy. In a preferred example, the disorder is AMD, preferably dry AMD.

In another example, the disclosure provides a method of treating or preventing age-related macular degeneration (AMD) comprising administering the AAV vector, cell or pharmaceutical composition of the disclosure to a subject in need thereof. The AMD may, for example, be dry AMD. In a preferred example, the AMD is dry AMD.

In another example, the disclosure provides a method of treating or preventing diabetic retinopathy comprising administering the AAV vector, cell or pharmaceutical composition of the disclosure to a subject in need thereof.

The subject may, for example, have been diagnosed with AMD or be at risk from acquiring AMD.

In a preferred example, the AAV vector, cell or pharmaceutical composition is administered intraocularly.

In one example, the AAV vector, cell or pharmaceutical composition is administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection.

In a particularly preferred example, the AAV vector, cell or pharmaceutical composition is administered to the eye of a subject by subretinal injection.

In another example, the disclosure provides the use of the AAV vector, cell or pharmaceutical composition of the disclosure for manufacturing a medicament for treating or preventing a complement-mediated disorder of the eye.

In one example, the disorder is associated with over-activity of the complement C3b feedback cycle and/or under-activity of the C3b breakdown cycle. In one example, the disorder is age-related macular degeneration (AMD) or diabetic retinopathy. In a preferred example, the disorder is AMD, preferably dry AMD.

In another example, the disclosure provides the use of the AAV vector, cell or pharmaceutical composition of the disclosure for manufacturing a medicament for treating or preventing age-related macular degeneration (AMD). In a preferred example, the AMD is dry AMD.

In another example, the disclosure provides the use of the AAV vector, cell or pharmaceutical composition of the disclosure for manufacturing a medicament for treating or preventing diabetic retinopathy.

In one example, the AAV vector of the disclosure does not comprise a hAAT promoter.

In one example, the AAV vector of the disclosure does not comprise an ApoR enhancer.

In another example, the AAV vector of the disclosure does not comprise two ApoR enhancers.

In one example, the AAV vector of the disclosure does not comprise an AAV2 genome and an AAV8 capsid protein, i.e. the AAV vector of the disclosure is not an AAV2/8 vector.

In one example, the AAV vector, cell or pharmaceutical composition of the disclosure is not administered systemically. In another example, the AAV vector, cell or pharmaceutical composition of the disclosure is not administered intravenously.

DESCRIPTION OF THE DRAWINGS

Figure 1

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

Figure 2

An agarose gel of restriction digests of CFI and CFIco. The 1752 bp band of CFI was excised and cloned into the pAAV-CBA- WPRE-bGHpA backbone.

Figure 3

Immunoblotting of CFI (Figure 3A) and of GFP (Figure 3B). 3A: CFI appears as a 70kDa band (non-reduced) and was expressed at equal rates after transfection of ARPE-19 with pAAV.CFI or pAAV.CFIco. No CFI was expressed after transfection with pAAV. 10% normal human serum (NHS) was used as a positive control for CFI immunoblotting. 3B: Transfection efficiency was analysed by co-transfection of ARPE-19 cells with pCMV.GFP. GFP appears as a 30kDa band and the immunoblot confirmed that cells have been transfected at similar efficiencies.

Figure 4

Immunoblotting of CFI in supernatant of virus transduced HEK-293 and ARPE-19 cell lines. 4A: Supernatant was loaded under non-reducing conditions and CFI was detected with a mouse monoclonal antibody to human CFI (OX21, Thermo Fisher Scientific) and a donkey anti-mouse IgG HRP conjugated antibody (Abcam). CFI and CFIco were expressed in both cell lines. Transduction of cell lines with AAV.GFP served as a negative control while 0.5µg of plasma purified human CFI (called "CFIpl" herein) (Comptech) served as a positive

CLAIMS

1. An adeno-associated viral (AAV) vector comprising a nucleotide sequence encoding Factor I or a fragment or derivative thereof, wherein the Factor I, fragment, or derivative is capable of cleaving C3b into iC3b.

2. The AAV vector of claim 1, wherein the nucleotide sequence encoding Factor I or fragment or derivative thereof comprises a sequence selected from the group consisting of:

   (a) a nucleotide sequence encoding an amino acid sequence that has at least 70% identity to SEQ ID NO: 1 or 9;

   (b) a nucleotide sequence that has at least 70% identity to SEQ ID NO: 2 or 8; and

   (c) the nucleotide sequence of SEQ ID NO: 2 or 8.

3. The AAV vector of claim 1 or 2, wherein the viral vector is in the form of a viral particle.

4. The AAV vector of claim 3, wherein the AAV viral particle comprises an AAV2 genome and AAV2 capsid proteins.

5. The AAV vector of any preceding claim, wherein the nucleotide sequence encoding Factor I or fragment or derivative thereof is operably linked to a CAG promoter, preferably a promoter with the nucleotide sequence of SEQ ID NO: 5.

6. An isolated cell transfected with the AAV vector of any one of claims 1-5.

7. A pharmaceutical composition comprising the AAV vector of any one of claims 1-5 or the cell of claim 6 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

8. The AAV vector, cell or pharmaceutical composition of any one of claims 1-7 for use in treating or preventing a complement-mediated disorder of the eye, wherein the disorder is age-related macular degeneration (AMD) or diabetic retinopathy.

9. The AAV vector, cell or pharmaceutical composition for use according to claim 8, wherein the disorder is AMD.

10. The AAV vector, cell or pharmaceutical composition for use according to claim 9, wherein the AMD is dry AMD.

11. The AAV vector, cell or pharmaceutical composition for use according to claim 10, wherein the formation of geographic atrophy is prevented or reduced, and/or the amount of geographic atrophy is reduced.

12. The AAV vector, cell or pharmaceutical composition for use according to claim 10, wherein the progression of geographic atrophy is slowed.

13. The AAV vector, cell or pharmaceutical composition for use according to claim 12, 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.

14. The AAV vector, cell or pharmaceutical composition for use according to any one of claims 8-13, wherein the AAV vector, cell or pharmaceutical composition to be administered increases the level of C3b-inactivating and iC3b-degradation activity in a subject, or in an eye, such as in the retinal pigment epithelium (RPE), of a subject, optionally to a level that exceeds a normal level in a subject, or eye or RPE thereof.

15. The AAV vector, cell or pharmaceutical composition for use according to any one of claims 8-14, wherein the AAV vector, cell or pharmaceutical composition is to be administered intraocularly.

16. The AAV vector, cell or pharmaceutical composition for use according to any one of claims 8-15, wherein the AAV vector, cell or pharmaceutical composition is to be administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection.

17. The AAV vector, cell or pharmaceutical composition for use according to any one of claims 8-16, wherein the AAV vector, cell or pharmaceutical composition is to be administered to the eye of a subject by subretinal injection.

18. Use of the AAV vector, cell or pharmaceutical composition of any one of claims 1-7 in the manufacture of a medicament for treating or preventing a complement-mediated disorder of the eye, wherein the disorder is age-related macular degeneration (AMD) or diabetic retinopathy.

19. The use of claim 18, wherein the disorder is AMD.

20. The use of claim 19, wherein the AMD is dry AMD.

21. The use of claim 20, wherein the formation of geographic atrophy is prevented or reduced, and/or the amount of geographic atrophy is reduced.

22. The use of claim 20, wherein the progression of geographic atrophy is slowed.

23. The use of claim 22, 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.

24. The use of any one of claims 18-23, wherein the AAV vector, cell or pharmaceutical composition to be administered increases the level of C3b-inactivating and iC3b-degradation activity in a subject, or in an eye, such as in the retinal pigment epithelium (RPE), of a subject, optionally to a level that exceeds a normal level in a subject, or eye or RPE thereof.

25. The use of any one of claims 18-24, wherein the AAV vector, cell or pharmaceutical composition is to be administered intraocularly.

26. The use of any one of claims 18-25, wherein the AAV vector, cell or pharmaceutical composition is to be administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection.

27. The use of any one of claims 18-26, wherein the AAV vector, cell or pharmaceutical composition is to be administered to the eye of a subject by subretinal injection.