

[0006] Current standard of care anti-VEGF agents need to be re-administered via intravitreal injection (IVT) injection every 4 to 8 weeks to achieve optimal therapeutic outcomes and maintain visual acuity. Compliance with such a regimen is burdensome to patients, their caregivers, and the healthcare system, and most patients fall out of compliance with the optimal regimen over time, which is correlated with vision loss (Khanani AM, *et al.*). In addition, there are complications including endophthalmitis, retinal detachments, traumatic cataract, and elevated intraocular pressure (IOP); the risk of these complications are likely to increase with repeated IVT injections (Falavarjani *et al.*, (2013) Eye (Lond), 27(7):787-794).

[0007] Therefore, there is a need in the art for therapies for ocular neovascular diseases such as wAMD that are effective, reduce the risk of adverse effects, and are amenable to high long-term patient compliance.

SUMMARY-OF-THE-DISCLOSURE

[0008] In one aspectexample, provided herein is a method for treating an ocular neovascular disease in an individual, the method comprising administering a unit dose of about 6×10^{11} vector genomes (vg) or less of recombinant adeno-associated virus (rAAV) particles to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise: (a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and (b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO. 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodimentexamples, the method comprises reducing retinal fluid in an eye of the individual.

[0008a] In one aspect, the present disclosure refers to use of a unit dose of about 6×10^{11} vector genomes (vg) or less of recombinant adeno-associated virus (rAAV) particles in the manufacture of a medicament for treating an ocular neovascular disease in an individual, wherein the unit dose of rAAV particles is to be administered to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise: a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein.

[0009] In another aspectexample, provided herein is a method for reducing retinal fluid in an eye of an individual with an ocular neovascular disease, the method comprising administering a unit dose of rAAV particles to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise: (a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and (b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO. 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some embodimentexamples, the individual has received at least one treatment of an anti-VEGF agent in about last 12 weeks prior to administration of the unit dose of rAAV particles. In some embodimentexamples, the amount or presence of retinal fluid in the one eye of the individual is refractory to prior treatment with an anti-VEGF agent. In some embodimentexamples, the anti-VEGF agent is aflibercept. In some embodimentexamples, the retinal fluid in the one eye is reduced by at least about 60%. In some embodimentexamples, the retinal fluid in the one eye is reduced by about 80% compared to the level of retinal fluid in the one eye of the individual prior to administration of the rAAV to the individual. In some embodimentexamples, the retinal fluid is subretinal fluid (SRF) or intraretinal fluid (IRF). In

some embodimentexamples, the unit dose of rAAV particles is about 6×10^{11} vector genomes per eye (vg/eye) or less.

[0009a] In one aspect, the present disclosure refers to use of a unit dose of rAAV particles in the manufacture of a medicament for reducing retinal fluid in an eye of an individual with an ocular neovascular disease, wherein the unit dose of rAAV particles is to be administered to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise: a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein.

[0010] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the unit dose of rAAV particles is about 6×10^{10} to about 2×10^{11} vector genomes per eye (vg/eye). In some embodimentexamples, the unit dose of rAAV particles is about 2×10^{11} or about 6×10^{10} vector genomes per eye (vg/eye).

[0011] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the methods provided herein further comprise administering a unit dose of rAAV particles to the contralateral eye of the individual. In some embodimentexamples, the administering the unit dose of rAAV particles to the contralateral eye is up to about 2 weeks after administering the unit dose of rAAV particles to the one eye. In some embodimentexamples, the unit dose of rAAV particles administered to the contralateral eye of the individual comprises the same or less vector genomes per eye (vg/eye) than the unit dose of rAAV particles administered to the one eye of the individual. In some embodimentexamples, the administering the unit dose of rAAV particles to the contralateral eye is at least about 2 weeks after administering the unit dose of rAAV particles to the one eye. In some embodimentexamples, the unit dose of rAAV particles administered to the contralateral eye of the individual comprises more vector genomes per eye (vg/eye) than the unit dose of rAAV particles administered to the one eye of the individual.

[0012] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the polypeptide comprises the amino acid sequence of SEQ ID NO: 35. In some embodimentexamples, the polypeptide is aflibercept.

[0013] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the nucleic acid further comprises a first enhancer region, a promoter region, a 5'UTR region, a second enhancer region, and a polyadenylation site. In some embodimentexamples, the nucleic acid comprises, in the 5' to 3' order: (a) a first enhancer region; (b) a promoter region; (c) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs); (d) a 5'UTR region; (e) a second enhancer region; and (f) a polyadenylation site. In some embodimentexamples, the first enhancer region comprises a CMV sequence comprising the sequence of SEQ ID NO: 22 or a sequence having at least 85% identity thereto. In some embodimentexamples, the promoter region comprises a CMV sequence comprising the sequence of SEQ ID NO: 23 or a sequence having at least 85% identity thereto. In some embodimentexamples, the polypeptide comprises the amino acid sequence of SEQ ID NO: 35 or a sequence having at least 85% identity thereto. In some embodimentexamples, the polypeptide is aflibercept. In some embodimentexamples, the 5'UTR region comprises, in 5' to 3' order, a TPL sequence comprising the sequence of SEQ ID NO: 24 or a sequence having at least 85% identity thereto, and an eMLP sequence comprising the sequence of SEQ ID NO: 25 or a sequence having at least 85% identity thereto. In some embodimentexamples, the second enhancer region comprises a full EES sequence comprising the sequence of SEQ ID NO: 26 or a sequence having at least 85% identity thereto. In some embodimentexamples, the polyadenylation site comprises a HGH polyadenylation site comprising

the sequence of SEQ ID NO: 27 or a sequence having at least 85% identity thereto. In some [embodimentexamples](#), the nucleic acid further comprises (a) a first enhancer region comprising a CMV sequence comprising the sequence of SEQ ID NO: 22 or a sequence having at least 85% identity thereto; (b) a promoter region, comprising a CMV sequence comprising the sequence of SEQ ID NO: 23 or a sequence having at least 85% identity thereto; (c) a 5'UTR region comprising, in 5' to 3' order, a TPL sequence comprising the sequence of SEQ ID NO: 24 or a sequence having at least 85% identity thereto, and an eMLP sequence comprising the sequence of SEQ ID NO: 25 or a sequence having at least 85% identity thereto; (d) a second enhancer region comprising a full EES sequence comprising the sequence of SEQ ID NO: 26 or a sequence having at least 85% identity thereto; and (e) a HGH polyadenylation site comprising the sequence of SEQ ID NO: 27 or a sequence having at least 85% identity thereto. In some [embodimentexamples](#), the nucleic acid comprises AAV ITRs flanking the elements.

[0014] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the AAV2 capsid protein comprises the amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13. In some [embodimentexamples](#), the AAV2 capsid protein comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein. In some [embodimentexamples](#), the AAV2 capsid protein comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13.

[0015] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye is by intravitreal administration.

[0016] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the unit dose of rAAV particles is in a pharmaceutical formulation. In some [embodimentexamples](#), the pharmaceutical formulation comprises the rAAV particles, sodium chloride, sodium phosphate and a surfactant. In some [embodimentexamples](#), the pharmaceutical formulation comprises about 150 to about 200 mM sodium chloride, about 1 to about 10 mM monobasic sodium phosphate, about 1 to about 10 mM dibasic sodium phosphate, about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, and about 6×10^{13} to about 6×10^{10} vector genomes (vg) per mL (vg/mL) of the rAAV particles, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5. In some [embodimentexamples](#), the pharmaceutical formulation comprises about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 6×10^{12} vg/mL of the rAAV particles, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3. In some [embodimentexamples](#), the pharmaceutical formulation comprises about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 6×10^{11} vg/mL of the rAAV particles, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3.

[0017] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the unit dose of rAAV particles comprises a volume of about 25 μ L to about 250 μ L. In some [embodimentexamples](#), the unit dose of rAAV particles comprises a volume of about 100 μ L. In some [embodimentexamples](#), the unit dose of rAAV particles comprises a volume of about 30 μ L.

[0018] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the individual received prior treatment for the ocular neovascular disease with an anti-VEGF agent. In some [embodimentexamples](#), the anti-VEGF agent is aflibercept.

[0019] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the ocular neovascular disease is wet age-related macular degeneration (AMD), retinal neovascularization, choroidal neovascularization diabetic retinopathy, proliferative diabetic retinopathy, retinal vein occlusion, central retinal vein occlusion, branched retinal vein occlusion, diabetic macular edema, diabetic retinal ischemia, ischemic retinopathy, diabetic retinal edema, or any combination thereof.

[0020] In some [embodimentexample](#)s that may be combined with any of the preceding [embodimentexamples](#), the unit dose of rAAV particles is administered in combination with steroid treatment. In some [embodimentexamples](#), the steroid treatment is a corticosteroid treatment. In some [embodimentexamples](#), the steroid treatment is a systemic steroid treatment. In some [embodimentexamples](#), the steroid treatment is an oral steroid treatment. In some [embodimentexamples](#), the steroid treatment is a prednisone treatment. In some [embodimentexamples](#), the steroid treatment is a topical steroid treatment. In some [embodimentexamples](#), the steroid treatment is a difluprednate treatment. In some [embodimentexamples](#), the steroid is administered before, during and/or after administration of the unit dose of rAAV particles.

[0021] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the steroid treatment is a topical steroid treatment and the topical steroid treatment is a daily steroid treatment for up to about 4 weeks, up to about 6 weeks, or up to about 8 weeks from administering the unit dose of rAAV particles. In some [embodimentexamples](#), the topical steroid treatment comprises about four administrations of topical steroid on about week 1, about three administrations of topical steroid on about week 2, about two administrations of topical steroid on about week 3, and about one administration of topical steroid on about week 4; timing starting with and following administration of the unit dose of rAAV particles. In some [embodimentexamples](#), the topical steroid comprises difluprednate 0.05% at a dose of about 1 μ g to about 3 μ g. In some [embodimentexamples](#), the topical steroid comprises difluprednate 0.05% at a dose of about 2.5 μ g.

[0022] In some [embodimentexample](#)s that may be combined with any of the preceding [embodimentexamples](#), the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance or a decrease of retinal thickness compared to the retinal thickness prior to administration of the unit dose of rAAV particles. In some [embodimentexamples](#), the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in retinal thickness compared to the retinal thickness prior to administration of the unit dose of rAAV particles. In some [embodimentexamples](#), the decrease in retinal thickness is at least about 10% compared to the

retinal thickness prior to administration of the unit dose of rAAV particles. In some embodimentexamples, retinal thickness is central subfield thickness (CST) or central retinal thickness (CRT).

[0023] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance or a decrease in macular volume compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodimentexamples, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in macular volume compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodimentexamples, the decrease in macular volume is at least about 10% compared to the macular volume prior to administration of the unit dose of rAAV particles.

[0024] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance or an improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodimentexamples, wherein the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance or an improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodimentexamples, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodimentexamples, visual acuity is best corrected visual acuity (BCVA).

INCORPORATION BY REFERENCE

[0025] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0027] **FIGS. 1A-1B** provide schematics of the investigational medicinal product and the phase I study described in Examples 1 and 2. **FIG. 1A** is a schematic of AAV2.7m8-aflibercept. AAV2.7m8-aflibercept is a recombinant, replication-deficient adeno-associated viral (rAAV) vector containing the AAV2.7m8 protein capsid and a vector genome containing an expression cassette of a codon-optimized version of the aflibercept cDNA under the control of a ubiquitous chimeric promoter (C11). The AAV2.7m8-aflibercept vector genome also contains two AAV2 inverted terminal repeat sequences (ITR) flanking the aflibercept cDNA expression cassette. **FIG. 1B** is a diagram summarizing the study design for the phase I study described in Examples 1 and 2.

CLAIMS

1. ~~A method for treating an ocular neovascular disease in an individual, the method comprising administering~~Use of a unit dose of about 6×10^{11} vector genomes (vg) or less of recombinant adeno-associated virus (rAAV) particles in the manufacture of a medicament for treating an ocular neovascular disease in an individual, wherein the unit dose of rAAV particles is to be administered to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise:
 - a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and
 - b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein.
2. The ~~method-use~~ of claim 1, wherein ~~the~~ retinal fluid in the one eye of the individual with the ocular neovascular disease is reduced.
3. ~~A method for reducing retinal fluid in an eye of an individual with an ocular neovascular disease, the method comprising administering~~Use of a unit dose of rAAV particles in the manufacture of a medicament for reducing retinal fluid in an eye of an individual with an ocular neovascular disease, wherein the unit dose of rAAV particles is to be administered to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise:
 - a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and
 - b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein.
4. The ~~method-use~~ of claim 3, wherein:

the individual has received at least one treatment of an anti-VEGF agent in about 12 weeks prior to administration of the unit dose of rAAV particles; and/or

the amount or presence of retinal fluid in the one eye of the individual is refractory to prior treatment with an anti-VEGF agent;

optionally wherein the anti-VEGF agent is aflibercept.

5. ~~The method of claim 3 or claim 4, wherein the amount or presence of retinal fluid in the one eye of the individual is refractory to prior treatment with an anti-VEGF agent.~~

6. ~~The method of claim 4 or claim 5, wherein the anti-VEGF agent is aflibercept.~~

7.5. ~~The method-use~~ of any one of claims 2-64, wherein:

~~the retinal fluid in the one eye is reduced by at least about 60%;~~

~~the retinal fluid in the one eye is reduced by about 80% compared to the level of retinal fluid in the one eye of the individual prior to administration of the rAAV particles to the individual;~~

~~the retinal fluid is subretinal fluid (SRF) or intraretinal fluid (IRF); and/or~~

~~the unit dose of rAAV particles is about 6×10^{11} vector genomes per eye (vg/eye) or less.~~

8. ~~The method of any one of claims 2-7, wherein the retinal fluid in the one eye is reduced by about 80% compared to the level of retinal fluid in the one eye of the individual prior to administration of the rAAV to the individual.~~

9. ~~The method of any one of claims 2-8, wherein the retinal fluid is subretinal fluid (SRF) or intraretinal fluid (IRF).~~

10. ~~The method of any one of claims 2-9, wherein the unit dose of rAAV particles is about 6×10^{11} vector genomes per eye (vg/eye) or less.~~

11.6. ~~The method-use~~ of any one of claims 1-510, wherein:

~~the unit dose of rAAV particles is about 6×10^{10} to about 2×10^{11} vector genomes per eye (vg/eye); or~~

~~the unit dose of rAAV particles is about 2×10^{11} or about 6×10^{10} vector genomes per eye (vg/eye).~~

12. ~~The method of any one of claims 1-11, wherein the unit dose of rAAV particles is about 2×10^{11} or about 6×10^{10} vector genomes per eye (vg/eye).~~

13.7. ~~The method-use~~ of any one of claims 1-612, ~~wherein the unit dose of rAAV particles is to be further administered~~ further comprising administering a unit dose of rAAV particles to the contralateral eye of the individual.

14.8. ~~The method-use~~ of claim 713, wherein:

(a) ~~the administering the~~ unit dose of rAAV particles is to be administered to the contralateral eye ~~is~~ up to about 2 weeks after ~~administering administration of~~ the unit dose of rAAV particles to the one eye, optionally wherein the unit dose of rAAV particles administered to the contralateral eye of the individual comprises the same or less vector genomes per eye (vg/eye) than the unit dose of rAAV particles administered to the one eye of the individual; or

(b) the unit dose of rAAV particles is to be administered to the contralateral eye at least about 2 weeks after administration of the unit dose of rAAV particles to the one eye, optionally wherein the unit dose of rAAV particles administered to the contralateral eye of the individual comprises more vector genomes per eye (vg/eye) than the unit dose of rAAV particles administered to the one eye of the individual.

~~15. The method of claim 14, wherein the unit dose of rAAV particles administered to the contralateral eye of the individual comprises the same or less vector genomes per eye (vg/eye) than the unit dose of rAAV particles administered to the one eye of the individual.~~

~~16. The method of claim 13, wherein the administering the unit dose of rAAV particles to the contralateral eye is at least about 2 weeks after administering the unit dose of rAAV particles to the one eye.~~

~~17. The method of claim 16, wherein the unit dose of rAAV particles administered to the contralateral eye of the individual comprises more vector genomes per eye (vg/eye) than the unit dose of rAAV particles administered to the one eye of the individual.~~

~~18.9. The method use of any one of claims 1-8~~17, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 35, or wherein the polypeptide is aflibercept.

~~19. The method of any one of claims 1-18, wherein the polypeptide is aflibercept.~~

~~20.10. The method use of any one of claims 1-0,~~ wherein:

(a) the nucleic acid further comprises a first enhancer region, a promoter region, a 5'UTR region, a second enhancer region, and a polyadenylation site; optionally wherein the first enhancer region comprises a CMV sequence comprising the sequence of SEQ ID NO: 22 or a sequence having at least 85% identity thereto, the promoter region comprises a CMV sequence comprising the sequence of SEQ ID NO: 23 or a sequence having at least 85% identity thereto, the 5'UTR region comprises, in 5' to 3' order, a TPL sequence comprising the sequence of SEQ ID NO: 24 or a sequence having at least 85% identity thereto and an eMLP sequence comprising the sequence of SEQ ID NO: 25 or a sequence having at least 85% identity thereto, the second enhancer region comprises a full EES

sequence comprising the sequence of SEQ ID NO: 26 or a sequence having at least 85% identity thereto, and the polyadenylation site is an HGH polyadenylation site comprising the sequence of SEQ ID NO: 27 or a sequence having at least 85% identity thereto; or

(b) the nucleic acid comprises, in the 5' to 3' order:

(i) a first enhancer region, optionally wherein the first enhancer region comprises a CMV sequence comprising the sequence of SEQ ID NO: 22 or a sequence having at least 85% identity thereto,

(ii) a promoter region, optionally wherein the promoter region comprises a CMV sequence comprising the sequence of SEQ ID NO: 23 or a sequence having at least 85% identity thereto,

(iii) a 5'UTR region, optionally wherein the 5'UTR region comprises, in 5' to 3' order, a TPL sequence comprising the sequence of SEQ ID NO: 24 or a sequence having at least 85% identity thereto, and an eMLP sequence comprising the sequence of SEQ ID NO: 25 or a sequence having at least 85% identity thereto,

(iv) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35, optionally wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 35, or the polypeptide is aflibercept,

(v) a second enhancer region, optionally wherein the second enhancer region comprises a full EES sequence comprising the sequence of SEQ ID NO: 26 or a sequence having at least 85% identity thereto, and

(vi) a polyadenylation site, optionally wherein the polyadenylation site comprises a HGH polyadenylation site comprising the sequence of SEQ ID NO: 27 or a sequence having at least 85% identity thereto,

and flanked by AAV2 inverted terminal repeats (ITRs).

~~21. The method of any one of claims 1-20, wherein the nucleic acid comprises, in the 5' to 3' order:~~

~~(a) a first enhancer region;~~

~~(b) a promoter region;~~

~~(c) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35;~~

~~(d) a 5'UTR region;~~

~~(e) a second enhancer region; and~~

~~(f) a polyadenylation site;~~

~~and flanked by AAV2 inverted terminal repeats (ITRs).~~

22. ~~—The method of claim 20 or claim 21, wherein the first enhancer region comprises a CMV sequence comprising the sequence of SEQ ID NO: 22 or a sequence having at least 85% identity thereto.~~

23. ~~—The method of any one of claims 20-22, wherein the promoter region comprises a CMV sequence comprising the sequence of SEQ ID NO: 23 or a sequence having at least 85% identity thereto.~~

24. ~~—The method of any one of claims 20-23, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 35 or a sequence having at least 85% identity thereto.~~

25. ~~—The method of any one of claims 20-24, wherein the polypeptide is aflibercept.~~

26. ~~—The method of any one of claims 20-25, wherein the 5'UTR region comprises, in 5' to 3' order, a TPL sequence comprising the sequence of SEQ ID NO: 24 or a sequence having at least 85% identity thereto, and an eMLP sequence comprising the sequence of SEQ ID NO: 25 or a sequence having at least 85% identity thereto.~~

27. ~~—The method of any one of claims 20-26, wherein the second enhancer region comprises a full EES sequence comprising the sequence of SEQ ID NO: 26 or a sequence having at least 85% identity thereto.~~

28. ~~—The method of any one of claims 20-27, wherein the polyadenylation site comprises a HGH polyadenylation site comprising the sequence of SEQ ID NO: 27 or a sequence having at least 85% identity thereto.~~

29. The method of any one of claims 1-19, wherein the nucleic acid further comprises (a) a first enhancer region comprising a CMV sequence comprising the sequence of SEQ ID NO: 22 or a sequence having at least 85% identity thereto; (b) a promoter region, comprising a CMV sequence comprising the sequence of SEQ ID NO: 23 or a sequence having at least 85% identity thereto; (c) a 5'UTR region comprising, in 5' to 3' order, a TPL sequence comprising the sequence of SEQ ID NO: 24 or a sequence having at least 85% identity thereto, and an eMLP sequence comprising the sequence of SEQ ID NO: 25 or a sequence having at least 85% identity thereto; (d) a second enhancer region comprising a full EES sequence comprising the sequence of SEQ ID NO: 26 or a sequence having at least 85% identity thereto; and (e) a HGH polyadenylation site comprising the sequence of SEQ ID NO: 27 or a sequence having at least 85% identity thereto.

~~30.11.~~ The ~~method-use~~ of any one of claims 1-~~1029~~, wherein:

the AAV2 capsid protein comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein;

~~the AAV2 capsid protein comprises the amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13; or~~

~~the AAV2 capsid protein comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13.~~

~~31. The method of any one of claims 1-30, wherein the AAV2 capsid protein comprises the amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13.~~

~~32. The method of any one of claims 1-31, wherein the AAV2 capsid protein comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13.~~

~~33.12.~~ The ~~method-use~~ of any one of claims 1-~~1132~~, wherein the unit dose of rAAV particles is to be administered~~administration of the unit dose of rAAV particles~~ to the one eye and/or the contralateral eye ~~is~~ by intravitreal administration.

~~34.13.~~ The ~~method-use~~ of any one of claims 1-~~1233~~, wherein the unit dose of rAAV particles is in a pharmaceutical formulation.

~~35.14.~~ The ~~method-use~~ of claim ~~1334~~, wherein:

(a) the pharmaceutical formulation comprises the rAAV particles, sodium chloride, sodium phosphate and a surfactant;

(b) the pharmaceutical formulation comprises about 150 to about 200 mM sodium chloride, about 1 to about 10 mM monobasic sodium phosphate, about 1 to about 10 mM dibasic sodium phosphate, about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, and about 6×10^{13} to about 6×10^{10} vector genomes (vg) per mL (vg/mL) of the rAAV particles, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5; or

(c) the pharmaceutical formulation comprises about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 6×10^{12} vg/mL of the

rAAV particles, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3, or

(d) the pharmaceutical formulation comprises about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 6×10^{11} vg/mL of the rAAV particles, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3.

36. — ~~The method of claim 35, wherein the pharmaceutical formulation comprises about 150 to about 200 mM sodium chloride, about 1 to about 10 mM monobasic sodium phosphate, about 1 to about 10 mM dibasic sodium phosphate, about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, and about 6×10^{13} to about 6×10^{10} vector genomes (vg) per mL (vg/mL) of the rAAV particles, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5.~~

37. — ~~The method of claim 36, wherein the pharmaceutical formulation comprises about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 6×10^{12} vg/mL of the rAAV particles, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3.~~

38. ~~The method of claim 36, wherein the pharmaceutical formulation comprises about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 6×10^{11} vg/mL of the rAAV particles, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3.~~

39.15. ~~The method use of any one of claims 1-1438, wherein the unit dose of rAAV particles comprises a volume of: about 25 μ L to about 250 μ L, about 30 μ L, or about 100 μ L.~~

40. — ~~The method of claim 39, wherein the unit dose of rAAV particles comprises a volume of about 100 μ L.~~

41. ~~The method of claim 39, wherein the unit dose of rAAV particles comprises a volume of about 30 μ L.~~

42.16. ~~The method use of any one of claims 1-1541, wherein the individual received prior treatment for the ocular neovascular disease with an anti-VEGF agent, optionally wherein the anti-VEGF agent is aflibercept.~~

43. ~~The method of claim 42, wherein the anti-VEGF agent is aflibercept.~~

44.17. The ~~method-use~~ of any one of claims 1-1643, wherein the ocular neovascular disease is wet age-related macular degeneration (AMD), retinal neovascularization, choroidal neovascularization, diabetic retinopathy, proliferative diabetic retinopathy, retinal vein occlusion, central retinal vein occlusion, branched retinal vein occlusion, diabetic macular edema, diabetic retinal ischemia, ischemic retinopathy, diabetic retinal edema, or any combination thereof.

45.18. The ~~method-use~~ of any one of claims 1-1744, wherein the unit dose of rAAV particles is to be administered in combination with steroid treatment, optionally wherein:
the steroid treatment is a corticosteroid treatment or a prednisone treatment, and/or
the steroid treatment is to be administered before, during and/or after administration of the unit dose of rAAV particles.

~~46. The method of claim 45, wherein the steroid treatment is a corticosteroid treatment.~~

47.19. The ~~method-use~~ of claim ~~45 or claim 46~~18, wherein the steroid treatment is:

(a) a systemic steroid treatment and/or an oral steroid treatment; and/or

(b) a topical steroid treatment, optionally a difluprednate treatment.

~~48. The method of any one of claims 45-47, wherein the steroid treatment is an oral steroid treatment.~~

~~49. The method of any one of claims 45-48, wherein the steroid treatment is a prednisone treatment.~~

~~50. The method of claim 45 or claim 46, wherein the steroid treatment is a topical steroid treatment.~~

~~51. The method of claim 50, wherein the steroid treatment is a difluprednate treatment.~~

~~52. The method of any one of claims 45-51, wherein the steroid is administered before, during and/or after administration of the unit dose of rAAV particles.~~

53.20. The ~~method-use~~ of ~~claim 18 or claim 19~~any one of claims 50-52, wherein the steroid treatment is a topical steroid treatment, wherein:

~~(a) and~~ the topical steroid treatment is a daily steroid treatment for up to about 4 weeks, up to about 6 weeks, or up to about 8 weeks from ~~administering-administration of~~ the unit dose of rAAV particles; ~~or~~

~~(b) the topical steroid treatment comprises about four administrations of topical steroid on about week 1, about three administrations of topical steroid on about week 2, about two administrations of topical steroid on about week 3, and about one administration of topical steroid on about week 4; timing starting with and following administration of the unit dose of rAAV particles, optionally wherein the topical steroid treatment comprises difluprednate 0.05% at a dose of about 1 µg to about 3 µg, or at a dose of about 2.5 µg.~~

~~54. The method of claim 53, wherein the topical steroid treatment comprises about four administrations of topical steroid on about week 1, about three administrations of topical steroid on about week 2, about two administrations of topical steroid on about week 3, and about one administration of topical steroid on about week 4; timing starting with and following administration of the unit dose of rAAV particles.~~

~~55. The method of claim 54, wherein the topical steroid comprises difluprednate 0.05% at a dose of about 1 µg to about 3 µg.~~

~~56. The method of claim 54, wherein the topical steroid comprises difluprednate 0.05% at a dose of about 2.5 µg.~~

~~57.21. The method-use of any one of claims 1-2056, wherein administration of the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in:~~

~~(a) maintenance or a decrease in retinal thickness compared to the retinal thickness prior to administration of the unit dose of rAAV particles, a decrease in retinal thickness compared to the retinal thickness prior to administration of the unit dose of rAAV particles, or a decrease in retinal thickness of at least about 10% compared to the retinal thickness prior to administration of the unit dose of rAAV particles; optionally wherein retinal thickness is central subfield thickness (CST) or central retinal thickness (CRT); and/or~~

~~(b) maintenance or a decrease in macular volume compared to the macular volume prior to administration of the unit dose of rAAV particles, a decrease in macular volume compared to the macular volume prior to administration of the unit dose of rAAV particles, or a decrease in macular volume of at least about 10% compared to the macular volume prior to administration of the unit dose of rAAV particles; and/or~~

~~(c) maintenance or an improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles, or an improvement of visual acuity compared to the~~

visual acuity prior to administration of the unit dose of rAAV particles; optionally wherein visual acuity is best corrected visual acuity (BCVA).

~~58. — The method of claim 57, wherein the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in retinal thickness compared to the retinal thickness prior to administration of the unit dose of rAAV particles.~~

~~59. — The method of claim 57 or claim 58, wherein the decrease in retinal thickness is at least about 10% compared to the retinal thickness prior to administration of the unit dose of rAAV particles.~~

~~60. — The method of any one of claims 57-59, wherein retinal thickness is central subfield thickness (CST) or central retinal thickness (CRT).~~

~~61. — The method of any one of claims 1-60, wherein the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance or a decrease in macular volume compared to the macular volume prior to administration of the unit dose of rAAV particles.~~

~~62. — The method of claim 61, wherein the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a decrease in macular volume compared to the macular volume prior to administration of the unit dose of rAAV particles.~~

~~63. — The method of claim 62, wherein the decrease in macular volume is at least about 10% compared to the macular volume prior to administration of the unit dose of rAAV particles.~~

~~64. — The method of any one of claims 1-63, wherein the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance or an improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles.~~

~~65. — The method of any one of claims 1-64, wherein the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in an improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles.~~

~~66. — The method of any one of claims 64-65, wherein visual acuity is best corrected visual acuity (BCVA).~~