CLAIMS

1. Use of a unit dose of recombinant adeno-associated virus (rAAV) particles in the manufacture of a medicament for reducing fluctuation of central subfield thickness (CST) or central retinal thickness (CRT) in an individual, wherein the unit dose 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 use of claim 1, wherein:

the individual has received at least one treatment of an anti-VEGF agent in about 1 to 12 weeks prior to administration of the unit dose of rAAV particles; and/or

the level of fluctuation in the CST or CRT 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.

3. The use of claim 1, wherein:

the fluctuation level of CST or CRT after administration of rAAV particles in the one eye is reduced by about 40% to about 90% compared to the fluctuation level of CST or CRT in the one eye of the individual prior to administration of the rAAV to the individual,

optionally wherein the reduction is about 45% to about 85% or about 50% to about 90%.

4. The use of any one of claims 1-3, wherein the unit dose of rAAV particles is about 6 x 10^{10} to about 6 × 10^{11} vector genomes per eye (vg/eye) or less,

optionally wherein the rAAV particle is about 6 x 10^{10} vg/eye, about 2 x 10^{11} vg/eye, or about 6 x 10^{11} vg/eye.

5. The use of claims 1-4, wherein the unit dose of rAAV particles is to be administered to one eye of the individual after administration of the anti-VEGF agent to the one eye.

6. The use of claim 5, wherein:

(a) the unit dose of rAAV particles is to be administered to the one eye of the individual about 1 to 12 weeks after administration of the anti-VEGF agent, or administration of the anti-VEGF agent to the one eye of the individual on Day 1, and administration of the unit dose of rAAV particles to the one eye of the individual on Day 8; and/or

(b) the anti-VEGF agent comprises aflibercept, optionally wherein the aflibercept is to be administered at a dose of about 2 mg by intravitreal injection.

7. The use of any one of claims 1-6, wherein the unit dose of rAAV particles is:

(a) between about 6×10^{10} to about 6×10^{11} vector genomes per eye (vg/eye);

(b) between about 6×10^{10} to about 2×10^{11} vector genomes per eye (vg/eye); or

(c) between about 2×10^{11} to about 6×10^{11} vector genomes per eye (vg/eye) or

the unit dose of rAAV particles is about 6×10^{10} vg/eye, about 2×10^{11} vg/eye, or about 6×10^{11} vg/eye.

8. The use of any one of claims 1-7, wherein the individual has one or more symptoms of an ocular neovascular disease in the contralateral eye.

9. The use of any one of claims 1-8, wherein the unit dose of rAAV particles is to be further administered to the contralateral eye of the individual.

10. The use of claim 9, wherein:

(a) the unit dose of rAAV particles is to be administered to the contralateral eye up to about 2 weeks after administration of the unit dose of rAAV particles to the one eye, optionally wherein:

(i) the unit dose of rAAV particles is to be administered to the contralateral eye on the same day as the unit dose of rAAV particles administered to the one eye, or the unit dose of rAAV particles is to be administered to the contralateral eye between about 1 day to about 14 days after administration of the unit dose of rAAV particles to the one eye, and/or

(ii) 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.

11. The use of any one of claims 1-10, wherein

the nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 40 or a sequence having at least 85% identity thereto;

the polypeptide comprises the amino acid sequence of SEQ ID NO: 35 or SEQ ID NO: 41; or

the polypeptide is aflibercept.

12. The use of any one of claims 1-11, 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 comprises an HGH polyadenylation site comprising the sequence of SEQ ID NO: 27 or a sequence having at least 85% identity thereto;

(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 nucleic acid encoding the polypeptide comprises the nucleic acid sequence of SEQ ID NO: 40 or a sequence having at least 85% identity thereto, and further optionally wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 35, the amino acid sequence of SEQ ID NO: 41 or a sequence having at least 95% identity thereto, or 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); and/or

(c) the nucleic acid comprises the sequence of SEQ ID NO: 39 or a sequence having at least 85% identity thereto.

13. The use of any one of claims 1-12, 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.

14. The use of any one of claims 1-13, wherein the rAAV particles comprise an AAV2 VP1 capsid protein comprising a GH loop that comprises the amino acid sequence of SEQ ID NO: 38 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 38.

15. The use of any one of claims 1-14, wherein the unit dose of rAAV particles:

(a) is to be administered to the one eye and/or the contralateral eye by intravitreal administration; and/or

(b) is in a pharmaceutical formulation, optionally wherein the pharmaceutical formulation comprises the rAAV particles, sodium chloride, sodium phosphate and a surfactant, and further optionally wherein the pharmaceutical formulation comprises:

(i) 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;

(ii) 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;

(iii) about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 2×10^{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

(iv) 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.

16. The use of any one of claims 1-15, wherein the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye is in a volume of about 25 μL to about 250 μL, or

wherein the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye comprises a volume of about 100 μL or about 30 μL.

17. The use of any one of claims 1-16, wherein:

(a) the individual received prior treatment for the ocular neovascular disease with an anti-VEGF agent, optionally wherein the individual has received 1 or 2 injections of an anti-VEGF agent in the one eye and/or in the contralateral eye prior to administration of the rAAV particles in the one eye and/or in the contralateral eye; or

(b) the individual has not received prior treatment for the ocular neovascular disease with an anti-VEGF agent,

optionally wherein the anti-VEGF agent is aflibercept.

18. The use of any one of claims 1-17, 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.

19. The use of any one of claims 1-18, wherein the unit dose of rAAV particles is to be administered in combination with steroid treatment, optionally wherein the steroid treatment is a topical steroid treatment, and further optionally wherein the steroid treatment is a difluprednate treatment.

20. The use of claim 19, wherein the steroid is to be administered before, during and/or after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye.

21. The use of claim 19 or 20, wherein the steroid treatment is a topical steroid treatment, and wherein:

(a) 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 administration of the unit dose of rAAV particles;

(b) the topical steroid treatment comprises about four administrations of topical steroid per day on about week 1, about three administrations of topical steroid per day on about week 2, about two administrations of topical steroid per day on about week 3, and about one administration per day of topical steroid on about week 4; timing starting with and following administration of the unit dose of rAAV particles;

(c) the topical steroid treatment comprises about four administrations of topical steroid per day for about 3 weeks after administration of the unit dose of rAAV particles, followed by about 3 administrations of topical steroid per day for about 1 week, followed by about 2 administrations of topical steroid per day for about 1 week, and followed by about 1 administration of topical steroid per day for about 1 week; or

(d) the topical steroid treatment comprises about four administrations of topical steroid per day for about four weeks, followed by about three administrations of topical steroid per day for about one week, followed by about two administrations of topical steroid per day for about one week, and followed by about one administration of topical steroid per day for about one week; timing starting at about one week prior to administration of the unit dose of rAAV particles,

optionally wherein the topical steroid treatment comprises difluprednate 0.05% at a dose of about 1µg to about 3 µg, or at a dose of about 2.5µg.

22. The use of any one of claims 5-18, wherein the unit dose of rAAV particles is to be further administered in combination with a topical steroid treatment, optionally wherein:

the topical steroid treatment is a difluprednate treatment; and/or

the topical steroid treatment comprises about four administrations of topical steroid per day for about four weeks, followed by about three administrations of topical steroid per day for about one week, followed by about two administrations of topical steroid per day for about one week, and followed by about one administration of topical steroid per day for about one week; timing starting with and following administration of the anti-VEGF agent,

optionally wherein the topical steroid treatment comprises difluprednate 0.05% at a dose of about 1µg to about 3 µg, or at a dose of about 2.5µg.

23. The use of any one of claims 1-22, wherein the ocular neovascular disease is wet age-related macular degeneration (AMD).

24. The use of any one of claims 1-23, wherein the ocular neovascular disease is diabetic macular edema (DME).