CLAIMS

1. Use of a pharmaceutical composition comprising recombinant adeno-associated virus (rAAV) particles in the manufacture of a medicament for treating an ocular neovascular disease in an individual, wherein the medicament is for administration of a unit dose of about 1×10^{10} vector genomes (vg) to about 2×10^{11} vg of rAAV particles to one eye of the individual, wherein the medicament is to be administered by intravitreal injection (IVT) to the one eye of the individual, wherein the individual is a human, 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, and

wherein the medicament is for use in combination with an implant containing a steroid for the one eye.

2. The use of claim 1, wherein retinal fluid in the eye of the individual with an ocular neovascular disease is reduced.

3. Use of a pharmaceutical composition comprising 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 medicament is for administration of a unit dose of about 1×10^{10} vector genomes (vg) to about 2×10^{11} vg of rAAV particles to one eye of the individual, wherein the medicament is to be administered by intravitreal injection (IVT) to the one eye of the individual, wherein the individual is a human, 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, and
wherein the medicament is for use in combination with an implant containing a steroid in the one eye.

4. The use of claim 3, wherein: (a) 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; or (b) 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 use of any one of claims 2-4, wherein: (a) the retinal fluid in the one eye is reduced by at least about 60%; (b) 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; or (c) the retinal fluid is subretinal fluid (SRF) or intraretinal fluid (IRF).

6. The use of claim 4 or 5, wherein the medicament is for use in combination with administration of an anti-VEGF agent, wherein the medicament is to be administered after administration of the anti-VEGF agent.

7. The use of claim 6, wherein: (a) the unit dose of rAAV particles is to be administered to the one eye of the individual about 1 week or about 7 days after administration of the anti-VEGF agent; or (b) the anti-VEGF agent is to be administered to the one eye of the individual on Day 1, and the unit dose of rAAV particles is to be administered to the one eye of the individual on Day 8; or (c) the anti-VEGF agent comprises aflibercept, optionally wherein the aflibercept is to be administered at a dose of about 2 mg by intravitreal injection.

8. The use of any one of claims 1-7, wherein the unit dose of rAAV particles is: (a) between about 6×10^{10} to about 2×10^{11} vector genomes per eye (vg/eye); (b) about 2×10^{11} or about 6×10^{10} vector genomes per eye (vg/eye); (c) about 2×10^{11} vector genomes per eye (vg/eye); or (d) about 6×10^{10} vector genomes per eye (vg/eye).

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

10. The use of any one of claims 1-10, wherein a unit dose of rAAV particles is to be further administered to the contralateral eye of the individual.

11. The use of claim 10, wherein the unit dose of rAAV particles is to be administered to the contralateral eye up to about 2 weeks after the unit dose of rAAV particles to the one eye, optionally wherein 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 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 the unit dose of rAAV particles to the one eye, and optionally wherein the unit dose of rAAV particles to be 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 to be administered to the one eye of the individual.

12. The use of claim 10, wherein the unit dose of rAAV particles is to be administered to the contralateral eye at least about 2 weeks after the unit dose of rAAV particles to the one eye, optionally wherein the unit dose of rAAV particles to be administered to the contralateral eye of the individual comprises more vector genomes per eye (vg/eye) than the unit dose of rAAV particles to be administered to the one eye of the individual.

13. The use of any one of claims 1-12, wherein: (a) the nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 40 or a sequence having at least 85% identity thereto; (b) the polypeptide comprises the amino acid sequence of SEQ ID NO: 35 or SEQ ID NO: 41; or (c) the polypeptide is aflibercept.

14. The use of any one of claims 1-13, wherein 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 nucleic acid comprises, in the 5' to 3' order:
(a) a first enhancer region;
(b) a promoter region;
(c) a 5'UTR region;
(d) 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;
(e) a second enhancer region; and
(f) a polyadenylation site;
and flanked by AAV2 inverted terminal repeats (ITRs);
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; and
the promoter region comprises a CMV sequence comprising the sequence of SEQ ID NO: 23 or a sequence having at least 85% identity thereto.

15. The use of claim 14, wherein: (a) the nucleic acid encoding a polypeptide comprises the nucleic acid sequence of SEQ ID NO: 40 or a sequence having at least 85% identity thereto; (b) the polypeptide comprises the amino acid sequence of SEQ ID NO: 35 or a sequence having at least 95% identity thereto; (c) the polypeptide comprises the amino acid sequence of SEQ ID NO: 41 or a sequence having at least 95% identity thereto; or (d) the polypeptide is aflibercept.

16. The use of claim 14 or 15, 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; 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 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.

17. The use of any one of claims 1-13, 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; or
the nucleic acid comprises the sequence of SEQ ID NO: 39 or a sequence having at least 85% identity thereto; or
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; or
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; or

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.

18. The use of any one of claims 1-17, wherein the administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye is by intravitreal administration.

19. The use of any one of claims 1-18, wherein the unit dose of rAAV particles is in a pharmaceutical formulation, optionally wherein the pharmaceutical formulation comprises the rAAV particles, sodium chloride, sodium phosphate and a surfactant.

20. The use of claim 19, wherein: (a) 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;
(b) 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;
(c) the pharmaceutical formulation comprises 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
(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.

21. The use of any one of claims 1-20, wherein the unit dose of rAAV particles is to be administered to the one eye and/or to the contralateral eye in a volume of about 25 µL to about 250 µL, optionally wherein the unit dose of rAAV particles to be administered to the one eye and/or to the contralateral eye comprises a volume of about 100 µL or about 30 µL.

22. The use of any one of claims 1-21, wherein 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.

23. The use of any one of claims 1-3 or 5-21, wherein the individual has not received prior treatment for the ocular neovascular disease with an anti-VEGF agent.

24. The use of claim 22 or 23, wherein the anti-VEGF agent is aflibercept.

25. The use of any one of claims 1-24, 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.

26. The use of any one of claims 1-25, wherein administration of 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, optionally wherein the administration of 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, 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, and

wherein retinal thickness is central subfield thickness (CST) or central retinal thickness (CRT).

27. The use of any one of claims 1-26, wherein administration of 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, optionally wherein administration of 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, and 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.

28. The use of any one of claims 1-27, wherein administration of 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, optionally wherein administration of 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,
and wherein visual acuity is best corrected visual acuity (BCVA).

29. The use of any one of claims 1-28, wherein administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 50% of the individuals in the plurality not requiring an anti-VEGF rescue treatment, optionally wherein
at least about 50% of the individuals in the plurality do not require an anti-VEGF rescue treatment for at least about 20 weeks, at least about 36 weeks, at least about 52 weeks, at least about 56 weeks, or more after administration of the unit dose of rAAV particles.

30. The use of any one of claims 1-28, wherein administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 67% of the individuals in the plurality not requiring an anti-VEGF rescue treatment, optionally wherein
at least about 67% of the individuals in the plurality do not require an anti-VEGF rescue treatment for at least about 20 weeks, at least about 36 weeks, at least about 52 weeks, at least about 60 weeks, at least about 64 weeks, or at least about 66 weeks after administration of the unit dose of rAAV particles.

31. The use of any one of claims 1-28, wherein administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in at least about 78% of the individuals in the plurality not requiring an anti-VEGF rescue treatment, optionally wherein
at least about 78% of the individuals in the plurality do not require an anti-VEGF rescue treatment for at least about 20 weeks, at least about 36 weeks, or more after administration of the unit dose of rAAV particles.

32. The use of any one of claims 1-28, wherein administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in 100% of the individuals in the plurality not requiring an anti-VEGF rescue treatment, optionally wherein
100% of the individuals in the plurality do not require an anti-VEGF rescue treatment for at least about 64 weeks, at least about 68 weeks, at least about 72 weeks, at least about 76 weeks, at least about 80 weeks, at least about 84 weeks, or more after administration of the unit dose of rAAV particles.

33. The use of any one of claims 1-32, wherein administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of a plurality of individuals results in a reduction in the annualized anti-VEGF injection rate of at least about 80%, at least about 85%, at least about 87%, at least about 90%, at least about 95%, at least about 99%, or 100% compared to the annualized anti-VEGF injection rate prior to administration of the unit dose of rAAV particles.

34. The use of any one of claims 1-5, 8-25, or 26-33, wherein the ocular neovascular disease is wet age-related macular degeneration (AMD).

35. The use of any one of claims 1-3 or 6-28, wherein the ocular neovascular disease is diabetic macular edema (DME).

36. The use of claim 35, wherein administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in a 2-step or in a 3-step improvement in Diabetic Retinopathy Severity Scale (DRSS).

37. Use of a unit dose of about 1×10^{10} vector genomes (vg) to about 2×10^{11} vg of recombinant adeno- associated virus (rAAV) particles in the manufacture of a medicament for treating an ocular neovascular disease in an individual, wherein the medicament is to be administered by intravitreal injection (IVT) to one eye of the individual, wherein the individual is a human, 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, and
wherein the medicament is for use in combination with an implant containing a steroid in the one eye.

38. The use according to claim 37, wherein an anti-VEGF agent is to be administered to said one eye of the individual before administration of said unit dose.

39. The use of any one of claims 1-38, wherein the medicament is for further use in combination with an oral steroid treatment.

40. The use of claim 39, wherein the oral steroid treatment and the implant are corticosteroid treatments.

41. The use of claim 39 or 40, wherein the oral steroid treatment: (i) is a prednisone treatment,

(ii) is to be administered at a dose of:

a. less than about 0.8 mg/kg/day to about 1.00 mg/kg/day for the week prior to through week two following administration of the unit dose of rAAV particles,
b. less than about 0.59 mg/kg/day to about 0.75 mg/kg/day on weeks three to five following administration of the unit dose of rAAV particles,
c. less than about 0.4 mg/kg/day to about 0.6 mg/kg/day on week six after administration of the unit dose of rAAV particles,
d. less than about 0.25 mg/kg/day to about 0.375 mg/kg/day on week seven after administration of the unit dose of rAAV particles,
e. less than about 0.14 mg/kg/day to about 0.28 mg/kg/day on week eight after administration of the unit dose of rAAV particles, and
f. less than about 0.063 mg/kg/day to about 0.125 mg/kg/day on week nine after administration of the unit dose of rAAV particles,

(iii) is to be administered at a dose of:

a. less than or equal to about 1.00 mg/kg/day for the week prior to through week two following administration of the unit dose of rAAV particles,
b. less than or equal to about 0.75 mg/kg/day on weeks three to five following administration of the unit dose of rAAV particles,
c. less than or equal to about 0.6 mg/kg/day on week six after administration of the unit dose of rAAV particles,
d. less than or equal to about 0.375 mg/kg/day on week seven after administration of the unit dose of rAAV particles,
e. less than or equal to about 0.28 mg/kg/day on week eight after administration of the unit dose of rAAV particles, and
f. less than or equal to about 0.125 mg/kg/day on week nine after administration of the unit dose of rAAV particles, or

(iv) is to be administered at a dose of about 0.125 mg/kg/day to about 0.14 mg/kg/day on the first four days of week nine after administration of the unit dose of rAAV particles and about 0.0625 mg/kg/day to about 0.070 mg/kg/day on the first four days of week nine after administration of the unit dose of rAAV particles if the individual weighs 70 kg or more.

42. The use of claim 40, wherein the implant is a triamcinolone acetonide treatment or a dexamethasone treatment, optionally

wherein the implant is a triamcinolone acetonide treatment of about 2 mg, or

wherein the implant is to be administered one week prior to administration of the unit dose of rAAV particles and after administration of the unit dose of rAAV particles, or

wherein the implant comprises a dexamethasone of about 0.7 mg, or

wherein the implant is an intravitreal implant to be administered one week prior to administration of the unit does of rAAV particles.

43. The use of any one of claims 39-42, wherein the oral steroid treatment is to be administered before, during and/or after administration of the unit dose of rAAV particles.