

vessel growth, or proliferative DR (PDR), with new abnormal blood vessel growth within the retina or choroid. Diabetic macular edema (DME) is a complication of DR, and is another example of an ocular disease affecting the macula. DME affects up to 10% of people with diabetes and is caused by fluid accumulation in the macula. DME is the most frequent cause of sight loss in people with DR. Available therapies for treating DME include laser and anti-vascular endothelial growth factor (anti-VEGF) drugs such as aflibercept.

[0006] Aflibercept is a recombinant fusion protein that acts as a decoy receptor for vascular endothelial growth factor subtypes A and B (VEGF-A and VEGF-B) and placental growth factor (PGF). By binding to these ligands, aflibercept is able to prevent them from binding to vascular endothelial growth factor receptors (VEGFR), VEGFR-1 and VEGFR-2, to suppress neovascularization and decrease vascular permeability. Aflibercept consists of domain 2 of VEGFR-1 and domain 3 of VEGFR-2 fused with the Fc fragment of IgG1.

[0007] Current standard of care anti-VEGF agents such as aflibercept need to be re-administered via intravitreal (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 risks of these complications are likely to increase with repeated IVT injections (Falavarjani *et al.*, (2013) Eye (Lond), 27(7):787-794).

[0008] Therefore, there is a need in the art for therapies for ocular neovascular diseases such as wAMD, DR, or DME that are effective, reduce the risk of adverse effects, and are amenable to high long-term patient compliance.

SUMMARY-OF-THE-DISCLOSURE

[0009] 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, or consisting of, 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.

[0009a] 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 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 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, or consisting of, 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.

[\[0010a\] 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.](#)

[\[0011\]](#) In another [aspectexample](#), provided herein is a method for treating an ocular neovascular disease in an individual, the method comprising: (a) administering an anti-VEGF agent to one eye of the individual; and (b) administering a unit dose of about 6×10^{11} vector genomes (vg) or less of recombinant adeno-associated virus (rAAV) particles to the one eye of the individual after administration of the anti-VEGF agent, wherein the individual is a human, and wherein the rAAV particles comprise: (i) 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 (ii) 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 administering the unit dose of rAAV particles to the one eye of the individual about 1 week or about 7 days after administration of the anti-VEGF agent. In some [embodimentexamples](#), the method comprises administering the unit dose of rAAV particles to the one eye of the individual about 1 week to about 2 weeks after administration of the anti-VEGF agent. In some [embodimentexamples](#), the method comprises administering the unit dose of rAAV particles to the one eye of the individual about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, or about 15 days after administration of the anti-VEGF agent. In some [embodimentexamples](#), the method comprises administering the anti-VEGF agent to the one eye of the individual on Day 1, and administering the unit dose of rAAV particles to the one eye of the individual on Day 8. In some [embodimentexamples](#), the anti-VEGF agent comprises aflibercept. In some [embodimentexamples](#), the aflibercept is administered at a dose of about 2 mg by intravitreal injection. In some [embodimentexamples](#), the method further comprises administering a topical steroid treatment. In some [embodimentexamples](#), the topical steroid treatment is a difluprednate treatment. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the topical steroid treatment comprises about four administrations of topical steroid per day for about one month, followed by about three administrations of topical steroid per day for about one month, followed by about two administrations of topical steroid per day for about one month, and followed by about one administration of topical steroid per day for about one month; timing starting with and following administration of the anti-VEGF agent. In some [embodimentexamples](#), the topical steroid treatment comprises about four administrations of topical steroid per day for about one month, followed by about three administrations of topical steroid per day for about one month, followed by about two administrations of topical steroid per day for about one month, and followed by about one administration of topical steroid per day for about one month; timing starting with and following administration of the rAAV particles. In some [embodimentexamples](#), the topical steroid comprises difluprednate 0.05% at a dose of about 1 µg to about 3 µg. In some [embodimentexamples](#), the topical steroid comprises difluprednate 0.05% at a dose of about 2.5 µg.

[0011a] 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 in combination with an anti-VEGF agent, and 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, wherein the individual is a human, and wherein the rAAV particles comprise: (i) 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 (ii) 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.

[0012] 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). In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the unit dose of rAAV particles is between about 6×10^{10} to about 6×10^{11} vector genomes per eye (vg/eye). In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the unit dose of rAAV particles is between about 6×10^{10} to about 2×10^{11} vector genomes per eye (vg/eye). In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the unit dose of rAAV particles is between about 2×10^{11} to about 6×10^{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). In some [embodimentexamples](#), the unit dose of rAAV particles is about 2×10^{11} vg/eye. In some [embodimentexamples](#), the unit dose of rAAV particles is about 6×10^{11} vg/eye.

[0013] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the individual has one or more symptoms of an ocular neovascular disease in the contralateral eye.

[0014] 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 administering the unit dose of rAAV particles to the contralateral eye is on the same day as the administering the unit dose of rAAV particles to the one eye; or the administering the unit dose of rAAV particles to the contralateral eye is between about 1 day to about 14 days 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.

[0015] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 40 or a sequence having at least 85% identity thereto.

[0016] 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](#) that may be combined with any of the preceding [embodimentexamples](#), the polypeptide comprises the amino acid sequence of SEQ ID NO: 41. In some [embodimentexamples](#), the polypeptide is aflibercept.

[0017] 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 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). 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 nucleic acid encoding a polypeptide comprises the nucleic acid sequence of SEQ ID NO: 40 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 comprises the amino acid sequence of SEQ ID NO: 41 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.

[0018] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the nucleic acid comprises the sequence of SEQ ID NO: 39 or a sequence having at least 85% identity thereto.

[0019] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), 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. In some [embodimentexamples](#), the rAAV particles comprise an AAV2 VP1 capsid protein comprising a GH loop that comprises an amino acid sequence having any of at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 38.

[0020] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the rAAV particles comprise an AAV2 VP1 capsid protein comprising the amino acid sequence of SEQ ID NO: 37 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 37. In some [embodimentexamples](#), the rAAV particles comprise an AAV2 VP1 capsid protein comprising an amino acid sequence having any of at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 37.

[0021] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the AAV2 capsid protein comprises, or consists of, 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. 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.

[0022] 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.

[0023] 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 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. 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.

[0024] 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. In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye comprises a volume of about 25 μ L to about 250 μ L. In some [embodimentexamples](#), the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye comprises a volume of about 100 μ L. In some [embodimentexamples](#), the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye comprises a volume of about 30 μ L.

[0025] 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 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. In some [embodimentexamples](#), the individual has not received prior treatment for the ocular neovascular disease with an anti-VEGF agent. In some [embodimentexamples](#), the anti-VEGF agent is aflibercept.

[0026] 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.

[0027] In some [embodimentexamples](#) 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 oral prednisone treatment comprises administering prednisone at a dose of about 60 mg per day for a total of 6 days starting at 3 days before administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye, followed by administering prednisone at a dose of about 40 mg per day for a total of 3 days, followed by administering prednisone at a dose of about 20 mg per day for a total of 2 days, and followed by administering prednisone at a dose of about 10 mg per day for a total of 2 days. 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. In some [embodimentexamples](#), the steroid is administered before, during and/or after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye.

[0028] 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 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. In some [embodimentexamples](#), 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. In some [embodimentexamples](#), the topical steroid treatment comprises about four administrations of topical steroid per day for about one month, followed by about three administrations of topical steroid per day for about one month, followed by about two administrations of topical steroid per day for about one month, and

followed by about one administration of topical steroid per day for about one month; timing starting at about the administration of the unit dose of rAAV particles. In some [embodimentexamples](#), the topical steroid treatment comprises about four administrations of topical steroid per day for about one month, followed by about three administrations of topical steroid per day for about one month, followed by about two administrations of topical steroid per day for about one month, and followed by about one administration of topical steroid per day for about one month; timing starting at about one week prior to administration of the unit dose of rAAV particles. In some [embodimentexamples](#), the topical steroid comprises difluprednate 0.05% at a dose of about 1µg to about 3 µg. In some [embodimentexamples](#), the topical steroid comprises difluprednate 0.05% at a dose of about 2.5µg.

[0029] In some [embodimentexamples](#), the ocular neovascular disease is wet age-related macular degeneration (wAMD).

[0030] 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 of retinal thickness compared to the retinal thickness prior to administration of the unit dose of rAAV particles. In some [embodimentexamples](#), the maintenance or the decrease of retinal thickness compared to the retinal thickness prior to administration of the unit dose of rAAV particles is present at about 30 weeks, about 34 weeks, about 44 weeks, about 6 months, about 1 year, about 1.5 years, about 2 years, about 3 years, about 5 years, about 10 years, or more after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual. 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).

[0031] 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 maintenance or the decrease in macular volume compared to the macular volume prior to administration of the unit dose of rAAV particles is present at about 30 weeks, about 34 weeks, about 44 weeks, about 6 months, about 1 year, about 1.5 years, about 2 years, about 3 years, about 5 years, about 10 years, or more after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual. 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.

[0032] 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](#), the maintenance or the improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles is present at about 30 weeks, about 34 weeks, about 44 weeks, about 6 months, about 1 year, about 1.5 years, about 2 years, about 3 years, about 5 years, about 10 years, or more after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual. 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).

[0033] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual provides a therapeutic benefit (e.g., treatment of an ocular neovascular disease, reduction of retinal fluid, maintenance or a decrease of retinal thickness, maintenance or a decrease in macular volume, and/or maintenance or an improvement of visual acuity). In some [embodimentexamples](#), administration of a single unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual provides a therapeutic benefit that is present at about 30 weeks or more, about 34 weeks or more, about 44 weeks or more, about 6 months or more, about 1 year or more, about 1.5 years or more, or about 2 years, about 3 years, about 5 years, about 10 years, or more after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual.

[0034] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), 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. In some [embodimentexamples](#), 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.

[0035] In some [embodimentexamples](#) that may be combined with any of the preceding [embodimentexamples](#), administration of a single 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. In some [embodimentexamples](#), 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.

[0036] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, 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. In some embodimentexamples, 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.

[0037] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, 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. In some embodimentexamples, 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.

[0038] In some embodimentexamples that may be combined with any of the preceding embodimentexamples, 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.

[0039] In some embodimentexamples, the ocular neovascular disease is diabetic macular edema (DME). 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 2-step or in a 3-step improvement in Diabetic Retinopathy Severity Scale (DRSS). 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 2-step improvement in Diabetic Retinopathy Severity Scale (DRSS). 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 3-step improvement in Diabetic Retinopathy Severity Scale (DRSS).

INCORPORATION BY REFERENCE

[0040] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] 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:

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 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 retinal fluid in the one eye of the individual with an ocular neovascular disease is reduced.
3. ~~Use of 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 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 3-9, wherein the unit dose of rAAV particles is about 6×10^{11} vector genomes per eye (vg/eye) or less.~~

11.6. ~~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 A method for treating an ocular neovascular disease in an individual, wherein the unit dose of rAAV particles is to be administered in combination with an anti-VEGF agent, and 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 the method comprising:~~

~~(a) administering an anti-VEGF agent to one eye of the individual; and~~

~~(b) administering a unit dose of about 6×10^{11} vector genomes (vg) or less of recombinant adeno-associated virus (rAAV) particles to the one eye of the individual after administration of the anti-VEGF agent, wherein the individual is a human, and wherein the rAAV particles comprise:~~

~~(i) 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~~

~~(ii) 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.~~

~~12.7. The method-use of claim 644, wherein:~~

~~(a) comprising administering 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, and/or 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; 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.~~

~~13. The method of claim 11 or claim 12, comprising administering the anti-VEGF agent to the one eye of the individual on Day 1, and administering the unit dose of rAAV particles to the one eye of the individual on Day 8.~~

~~14. The method of any one of claims 11-13, wherein the anti-VEGF agent comprises aflibercept.~~

~~15. The method of claim 14, where the aflibercept is administered at a dose of about 2 mg by intravitreal injection.~~

~~16.8. The method-use of any one of claims 1-715, 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), between about 6×10^{10} to about 2×10^{11} vector genomes per eye (vg/eye), or between about 2×10^{11} to about 6×10^{11} vector genomes per eye (vg/eye); or~~

~~(b) about 2×10^{11} or about 6×10^{11} vector genomes per eye (vg/eye).~~

~~17. The method of any one of claims 1-16, wherein the unit dose of rAAV particles is between about 6×10^{10} to about 2×10^{11} vector genomes per eye (vg/eye).~~

~~18. The method of any one of claims 1-16, wherein the unit dose of rAAV particles is between about 2×10^{11} to about 6×10^{11} vector genomes per eye (vg/eye).~~

~~19. The method of claim 18, wherein the unit dose of rAAV particles is about 2×10^{11} or about 6×10^{11} vector genomes per eye (vg/eye).~~

~~20. The method claim 19, wherein the unit dose of rAAV particles is about 2×10^{11} vector genomes per eye (vg/eye).~~

21. ~~The method claim 19, wherein the unit dose of rAAV particles is about 6×10^{11} vector genomes per eye (vg/eye).~~

~~22.9. The method-use of any one of claims 1-821, wherein the individual has one or more symptoms of an ocular neovascular disease in the contralateral eye.~~

~~23.10. The method-use of any one of claims 1-922, wherein the unit dose of rAAV particles is to be further administeredfurther comprising administering a unit dose of rAAV particles to the contralateral eye of the individual.~~

~~24.11. The method-use of claim 1023, 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 theadministration 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.~~

~~25. The method of claim 24, wherein:~~

~~(a) the administering the unit dose of rAAV particles to the contralateral eye is on the same day as the administering the unit dose of rAAV particles to the one eye; or~~

~~(b) the administering the unit dose of rAAV particles to the contralateral eye is between about 1 day to about 14 days after administering the unit dose of rAAV particles to the one eye.~~

~~26. The method of claim 24 or claim 25, 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.~~

~~27. The method of claim 23, 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.~~

~~28. The method of claim 27, 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.~~

~~29.12. The method use of any one of claims 1-1128, 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;
and/or~~

~~the polypeptide is aflibercept.~~

~~30. The method of any one of claims 1-29, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 35.~~

~~31. The method of any one of claims 1-30, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 41.~~

~~32. The method of any one of claims 1-31, wherein the polypeptide is aflibercept.~~

~~33.13. The method use of any one of claims 1-1132, 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.

~~34. The method of any one of claims 1-33, 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).~~

35. — ~~The method of claim 33 or claim 34, 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.~~

36. — ~~The method of any one of claims 33-35, 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.~~

37. — ~~The method of any one of claims 34-36, wherein 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.~~

38. — ~~The method of any one of claims 34-37, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 35 or a sequence having at least 95% identity thereto.~~

39. — ~~The method of any one of claims 34-38, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 41 or a sequence having at least 95% identity thereto.~~

40. — ~~The method of any one of claims 34-39, wherein the polypeptide is aflibercept.~~

41. — ~~The method of any one of claims 33-40, 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.~~

42. — ~~The method of any one of claims 33-41, 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.~~

43. — ~~The method of any one of claims 33-42, 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.~~

44. — ~~The method of any one of claims 1-32, 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.~~

45. The method of any one of claims 1-44, wherein the nucleic acid comprises the sequence of SEQ ID NO: 39 or a sequence having at least 85% identity thereto.

~~46.14.~~ The ~~method-use~~ of any one of claims 1-~~1345~~, 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.~~

~~47. The method of any one of claims 1-46, 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.~~

~~48. The method of any one of claims 1-47, 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.~~

~~49.15.~~ The ~~method-use~~ of any one of claims 1-~~1446~~, 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.

~~50.16.~~ The ~~method-use~~ of any one of claims 1-~~1549~~, wherein the ~~administration of the~~ unit dose of rAAV particles:

~~(a) is to be administered~~ to the one eye and/or the contralateral eye ~~is~~ 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.

~~51. — The method of any one of claims 1–50, wherein the unit dose of rAAV particles is in a pharmaceutical formulation.~~

~~52. — The method of claim 51, wherein the pharmaceutical formulation comprises the rAAV particles, sodium chloride, sodium phosphate and a surfactant.~~

~~53. — The method of claim 52, 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.~~

~~54. — The method of claim 53, 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.~~

~~55. — The method of claim 53, wherein 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.

56. ~~The method of claim 53, 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.~~

~~57.17.~~ The ~~method-use~~ of any one of claims 1-~~16~~56, 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 μ L to about 250 μ L, about 100 μ L, or about 30 μ L.

~~58. — The method of claim 57, wherein the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye comprises a volume of about 100 μ L.~~

~~59. The method of claim 57, wherein the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye comprises a volume of about 30 μ L.~~

~~60.18.~~ The ~~method-use~~ of any one of claims 1-~~17~~59, 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.

~~61. — The method of claim 60, 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.~~

~~62. — The method of any one of claims 1-3 or 7-59, wherein the individual has not received prior treatment for the ocular neovascular disease with an anti-VEGF agent.~~

~~63. The method of any one of claims 60-62, wherein the anti-VEGF agent is aflibercept.~~

~~64.19.~~ The ~~method-use~~ of any one of claims 1-~~1863~~, 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.

~~65.20.~~ The ~~method-use~~ of any one of claims 1-~~510~~ or ~~816-1964~~, 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 to the one eye and/or to the contralateral eye.

~~66.~~ The method of claim 65, wherein the steroid treatment is a corticosteroid treatment.

~~67.21.~~ The ~~method-use~~ of claim ~~65 or claim 66~~~~20~~, wherein the steroid treatment is a systemic steroid treatment, an oral steroid treatment, and/or a topical steroid treatment, optionally wherein the topical steroid treatment is a difluprednate treatment.

~~68.~~—The method of any one of claims 65-67, wherein the steroid treatment is an oral steroid treatment.

~~69.~~—The method of any one of claims 65-68, wherein the steroid treatment is a prednisone treatment.

~~70.~~—The method of claim 65 or claim 66, wherein the steroid treatment is a topical steroid treatment.

~~71.~~—The method of claim 70, wherein the steroid treatment is a difluprednate treatment.

~~72.~~ The method of any one of claims 65-71, wherein the steroid is administered before, during and/or after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye.

~~73.22.~~ The ~~method-use~~ of ~~any one of claims 70-72~~claim 20 or claim 21, 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 administering the 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 1 µg to about 3 µg, or at a dose of about 2.5 µg.

~~74. — The method of claim 73, wherein 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.~~

~~75. — The method of claim 73, wherein 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.~~

~~76. The method of claim 73, wherein 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.~~

~~77.23. The method-use of any one of claims 644-1964, wherein the unit dose of rAAV particles is to be further administered in combination with ~~comprising administering~~ 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 1 µg to about 3 µg, or at a dose of about 2.5 µg.~~

~~78. The method of claim 77, wherein the topical steroid treatment is a difluprednate treatment.~~

~~79. The method claim 77 or claim 78, wherein 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.~~

~~80. The method of any one of claims 71-79, wherein the topical steroid comprises difluprednate 0.05% at a dose of about 1 µg to about 3 µg.~~

~~81. The method of any one of claims 71-80, wherein the topical steroid comprises difluprednate 0.05% at a dose of about 2.5 µg.~~

~~82.24. The method-use of any one of claims 1-2381, wherein:~~

~~(a) the administering administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in:~~

~~(i) maintenance or a decrease of 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),

(ii) 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

(iii) 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); and/or

(b) 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:

(i) 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,

(ii) 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,

(iii) 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,

(iv) 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, and/or

(v) 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.

83. — ~~The method of claim 82, 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.~~

84. — ~~The method of claim 83, 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.~~

85. — ~~The method of any one of claims 82-84, wherein retinal thickness is central subfield thickness (CST) or central retinal thickness (CRT).~~

86. — ~~The method of any one of claims 1-85, 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.~~

87. — ~~The method of claim 86, 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.~~

88. — ~~The method of claim 87, 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.~~

89. — ~~The method of any one of claims 1-88, 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.~~

90. — ~~The method of claim 89, 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.~~

91. — ~~The method of claim 89 or claim 90, wherein visual acuity is best corrected visual acuity (BCVA).~~

92. — ~~The method of any one of claims 1-91, 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.~~

93. — The method of claim 92, 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.

94. — The method of any one of claims 1-91, 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.

95. — The method of claim 94, 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.

96. — The method of any one of claims 1-91, 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.

97. — The method of claim 96, 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.

98. — The method of any one of claims 1-91, 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.

99. — The method of claim 98, 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.

100. — The method of any one of claims 1-99, 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.

~~101.25.~~ The ~~method-use~~ of any one of claims 1-~~510~~, ~~816-2276~~, or ~~80-10024~~, wherein the ocular neovascular disease is wet age-related macular degeneration (AMD).

~~102.26.~~ The ~~method-use~~ of any one of claims 1-3, ~~611-1964~~, or ~~2377-2483~~, ~~85-87~~, or ~~89-91~~, wherein the ocular neovascular disease is diabetic macular edema (DME), 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 2-step or in a 3-step improvement in Diabetic Retinopathy Severity Scale (DRSS).

~~103.—The method of claim 102, 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 2-step or in a 3-step improvement in Diabetic Retinopathy Severity Scale (DRSS).~~

~~104.—A unit dose of about 6×10^{11} vector genomes (vg) or less of recombinant adeno-associated virus (rAAV) particles for use in a method for treating an ocular neovascular disease in an individual, the method comprising administering said unit dose 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.~~

~~105.—A unit dose of rAAV particles for use in a method for reducing retinal fluid in an eye of an individual with an ocular neovascular disease, the method comprising administering said unit dose 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.~~

~~106. The unit dose for use according to claim 104 or claim 105 wherein said method further comprises administering an anti-VEGF agent to said one eye of the individual before administration of said unit dose.~~