



(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2019/11/18
(87) Date publication PCT/PCT Publication Date: 2021/03/18
(85) Entrée phase nationale/National Entry: 2022/02/11
(86) N° demande PCT/PCT Application No.: US 2019/062066
(87) N° publication PCT/PCT Publication No.: 2021/050094
(30) Priorités/Priorities: 2019/09/11 (US62/899,070);
2019/10/10 (US62/913,648)

(51) Cl.Int./Int.Cl. *A61K 38/00* (2006.01),
A61K 9/10 (2006.01), *A61P 27/02* (2006.01),
C07K 16/22 (2006.01), *C12N 15/86* (2006.01)
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(54) Titre : METHODES DE TRAITEMENT DE MALADIES NEOVASCULAIRES OCULAIRES A L'AIDE DE VARIANTS
D'AAV2 CODANT POUR L'AFLIBERCEPT
(54) Title: METHODS OF TREATING OCULAR NEOVASCULAR DISEASES USING AAV2 VARIANTS ENCODING
AFLIBERCEPT

(57) **Abrégé/Abstract:**

Provided are methods for treating an ocular neovascular disease in an individual, comprising administering a unit dose of recombinant adeno-associated virus (rAAV) particles to an eye of the individual, 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

Date Submitted: 2022/02/11

CA App. No.: 3147843

Abstract:

Provided are methods for treating an ocular neovascular disease in an individual, comprising administering a unit dose of recombinant adeno-associated virus (rAAV) particles to an eye of the individual, 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

METHODS OF TREATING OCULAR NEOVASCULAR DISEASES USING AAV2 VARIANTS ENCODING AFLIBERCEPT

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No.62/899,070 filed September 11, 2019 and U.S. Provisional Patent Application No.62/913,648 filed October 10, 2019, the disclosure of each of which are hereby incorporated by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 627002001240SEQLIST.TXT, date recorded: November 18, 2019, size: 29 KB).

FIELD

[0003] The present disclosure relates to methods of treating ocular neovascular disease and disorders in an individual that comprise administering a single unit dose of a recombinant adeno associated virus (rAAV) particles encoding an anti-VEGF agent (e.g., aflibercept) to an eye of an individual.

BACKGROUND

[0004] Age-related macular degeneration (AMD) is a degenerative ocular disease affecting the macula, a light sensitive, small area in the center of the retina that is responsible for reading and fine vision. Conditions affecting the macula reduce central vision while leaving peripheral vision intact. In severe cases, the disease can lead to central blindness. AMD is a notable cause of vision loss in the US population among persons 65 years and older, and the estimated prevalence of any AMD among persons over 40 years of age is approximately 6.5% (Klein *et al.*, (2011) Arch Ophthalmol, 129(1):75-80). Neovascular or exudative or wet AMD (nAMD, wAMD, or nwAMD) is an advanced form of AMD. The hallmark of wAMD is choroidal neovascularization (CNV), which is the infiltration of abnormal blood vessels in the retina from the underlying choroid layer, resulting in retinal cell damage and central blindness. This abnormal angiogenic process is modulated by growth factors, in particular, vascular endothelial growth factor (VEGF).

[0005] The standard of care of wAMD is a class of molecules that bind to and sequester VEGF, such as ranibizumab (Lucentis) and aflibercept (Eylea). For example, 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.

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

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

SUMMARY OF THE DISCLOSURE

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

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

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

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

[0011] In some embodiments that may be combined with any of the preceding embodiments, the methods provided herein further comprise administering a unit dose of rAAV particles to the contralateral eye of the individual. In some embodiments, 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 embodiments, 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 embodiments, 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 embodiments, the unit dose of rAAV particles administered to the contralateral eye of the individual comprises more vector genomes per eye (vg/eye) than the unit dose of rAAV particles administered to the one eye of the individual.

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

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

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

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

[0016] In some embodiments that may be combined with any of the preceding embodiments, the unit dose of rAAV particles is in a pharmaceutical formulation. In some embodiments, the pharmaceutical formulation comprises the rAAV particles, sodium chloride, sodium phosphate and a surfactant. In some embodiments, 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 embodiments, 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 embodiments, the pharmaceutical formulation comprises about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 6×10^{11} vg/mL of the rAAV particles, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3.

[0017] In some embodiments that may be combined with any of the preceding embodiments, the unit dose of rAAV particles comprises a volume of about 25 μL to about 250 μL . In some embodiments, the unit dose of rAAV particles comprises a volume of about 100 μL . In some embodiments, the unit dose of rAAV particles comprises a volume of about 30 μL .

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

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

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

[0021] In some embodiments that may be combined with any of the preceding embodiments, 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 embodiments, 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 embodiments, the topical steroid comprises difluprednate 0.05% at a dose of about 1 μg to about 3 μg . In some embodiments, the topical steroid comprises difluprednate 0.05% at a dose of about 2.5 μg .

[0022] In some embodiments that may be combined with any of the preceding embodiments, 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 embodiments, 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 embodiments, 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 embodiments, retinal thickness is central subfield thickness (CST) or central retinal thickness (CRT).

[0023] In some embodiments that may be combined with any of the preceding embodiments, 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 embodiments, 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 embodiments, the decrease in macular volume is at least about 10% compared to the macular volume prior to administration of the unit dose of rAAV particles.

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

INCORPORATION BY REFERENCE

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

BRIEF DESCRIPTION OF THE DRAWINGS

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

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

[0028] FIGS. 2A-2L show optical coherence tomography (OCT) images and retinal thickness maps derived from OCT images taken from subjects in Cohort 1 of the study described in Example 1. The OCT images were taken at the indicated times before and after administration of AAV2.7m8-aflibercept (Day 1). The anti-VEGF IVT treatment interval is indicated for all subjects. **FIG. 2A** provides OCT images and retinal thickness maps derived from OCT images taken from Subject 1 at five office visits at the times indicated prior to the Screening aflibercept injection. OCT images were taken immediately prior to treatment with aflibercept standard of care. Subject 1 required aflibercept IVT every 5-7 weeks and exhibited refractory subretinal fluid and a pigment epithelial detachment (PED) despite aflibercept standard of care treatment. **FIG. 2B** provides OCT images and retinal thickness maps derived from OCT images taken from Subject 1 at the Screening aflibercept injection (Day -7), at the AAV2.7m8-aflibercept injection (Day 1), and at follow-up visits at the times indicated. Subject 1 did not require any rescue injections after the AAV2.7m8-aflibercept injection. Subject 1 exhibited resolution of subretinal fluid beginning at week 4 remained free of subretinal and intraretinal fluid (remained dry). **FIG. 2C** provides OCT images and retinal thickness maps derived from OCT images taken from Subject 2 at five office visits at the times indicated prior to the Screening aflibercept injection. OCT images were taken immediately prior to treatment with aflibercept standard of care. Subject 2 required six aflibercept IVT treatments in the 8 months prior to AAV2.7m8-aflibercept treatment to maintain retinal anatomy. **FIG. 2D** provides OCT images and retinal thickness maps derived from OCT images taken from Subject 2 at the Screening aflibercept injection (Day -7), at the AAV2.7m8-aflibercept injection (Day 1), and at follow-up visits at the times indicated. Subject 2 did not require any rescue injections after the AAV2.7m8-aflibercept injection. Subject 2 exhibited stable retinal anatomy with no subretinal or intraretinal fluid through week 24. **FIG. 2E** provides OCT images and retinal thickness maps derived from OCT images taken from Subject 3 at four office visits at the times indicated prior to the Screening aflibercept injection. OCT images taken on Week -27 are not shown. OCT images were taken immediately prior to treatment with aflibercept standard of care. Subject 3 exhibited subretinal fluid, which increased when the interval between aflibercept IVT was increased from 5 to 7 weeks. **FIG. 2F** provides OCT images and retinal thickness maps derived from OCT images taken from Subject 3 at the Screening aflibercept injection (Day -7), the AAV2.7m8-aflibercept injection (Day 1), and at follow-up visits at the times indicated. Subject 3 did not require any rescue injections after the AAV2.7m8-aflibercept injection. Subject 3 exhibited resolution of refractory subretinal fluid by week 8, and stable retinal anatomy through week 24. **FIG. 2G** provides OCT images and retinal thickness maps derived from OCT images taken from Subject 4 at five office visits at the times indicated prior to the Screening aflibercept injection. OCT images were taken immediately prior to treatment with ranibizumab 0.5 mg IVT standard of care. Subject 4 exhibited subretinal fluid that was refractory to ranibizumab IVT injections. **FIG. 2H** provides OCT images and retinal thickness maps derived from OCT images taken from Subject 4 at the Screening aflibercept injection (Day -14), the AAV2.7m8-aflibercept injection (Day 1), and at follow-up visits at the times indicated. Subject 4 did not require any rescue injections after the

AAV2.7m8-aflibercept injection. Subject 4 exhibited resolution of refractory subretinal fluid by week 8, and stable retinal anatomy through week 24. **FIG. 2I** provides OCT images and retinal thickness maps derived from OCT images taken from Subject 5 at five office visits at the times indicated prior to the Screening aflibercept injection. OCT images were taken immediately prior to treatment with aflibercept standard of care. **FIG. 2J** provides OCT images and retinal thickness maps derived from OCT images taken from Subject 5 at the Screening aflibercept injection (Day -14), the AAV2.7m8-aflibercept injection (Day 1), and at follow-up visits at the times indicated. Subject 5 did not require any rescue injections after the AAV2.7m8-aflibercept injection. Subretinal fluid and PEDs present at the time of AAV2.7m8-aflibercept treatment resolved overtime, and retinal anatomy remained stable and free of subretinal or intraretinal fluid through week 24. **FIG. 2K** provides OCT images and retinal thickness maps derived from OCT images taken from Subject 6 at five office visits at the times indicated prior to the Screening aflibercept injection. OCT images were taken immediately prior to treatment with either bevacizumab 1.5 mg IVT standard of care or ranibizumab 0.5 mg IVT standard of care, as indicated. The appearance of Subject 6's retina was consistent with polypoidal choroidal vasculopathy (PCV). **FIG. 2L** provides OCT images and retinal thickness maps derived from OCT images taken from Subject 6 at the Screening aflibercept injection (Day -10), the AAV2.7m8-aflibercept injection (Day 1), and at follow-up visits at the times indicated. Subject 6 did not require any rescue injections after the AAV2.7m8-aflibercept injection. Subject 6 did not exhibit any increase in subretinal fluid and achieved some anatomical improvement through week 24. The contralateral eye of Subject 6 received standard of care aflibercept injections every 4 weeks over the course of the trial, and exhibited similar retinal morphology to the AAV2.7m8-aflibercept-treated eye.

[0029] **FIG. 3** shows the change in mean central retinal thickness (CST) for subjects in Cohort 1 of the study described in Example 1 at the indicated time points. Error bars indicate the 90% confidence interval, calculated using the T-distribution. Baseline (BL) indicates the measurement taken prior to the Screening aflibercept injection 7 to 15 days (e.g., 7-14 days) prior to AAV2.7m8-aflibercept treatment on day 1. At 24 weeks post AAV2.7m8-aflibercept treatment, subjects on average exhibited a change in CRT of $-52.7 \mu\text{m}$ (90% CI -86.5, -18.8). BL = baseline; D = day; W = week. The day 1 visit occurred 7-14 days after the baseline visit.

[0030] **FIG. 4** shows the mean best corrected visual acuity (BCVA) measurements based on Early Treatment Diabetic Retinopathy Study (ETDRS) letters assessments for subjects in Cohort 1 of the study described in Example 1 at the indicated time points. Error bars indicate the 90% confidence interval, calculated using the T-distribution. Baseline (BL) indicates the measurement taken prior to the Screening aflibercept injection 7 to 15 days (e.g., 7-14 days) prior to AAV2.7m8-aflibercept treatment on day 1. At 24 weeks post AAV2.7m8-aflibercept treatment, subjects on average exhibited a change in BCVA of -2 letters (90% CI -9.1, 5.1). BL = baseline; D = day; W = week. The day 1 visit occurred 7-14 days after the baseline visit.

[0031] **FIG. 5** provides the nucleic acid sequence of aflibercept (SEQ ID NO: 36).

[0032] FIG. 6 shows plots of anterior chamber cell and vitreous cell counts following treatment with AAV2.7m8-aflibercept for subjects 1-6 of the study described in Example 1. The steroid treatment administered to each patient is indicated below each plot. Aqueous cell count categories were based on the Standardization of Uveitis Nomenclature (SUN) criteria (Jabs, DA *et al.*, J Ophthalmol. 2005;140:509–516). Vitreous cell count categories were based on National Institutes of Health (NIH) guidelines. For aqueous cells, a cell count value of 0.5+ indicates 1-5 cells; a cell count value of 1+ indicates 6-15 cells; a cell count value of 2+ indicates 16-25 cells; a cell count value of 3+ indicates 26-50 cells; and a cell count value of 4+ indicates >50 cells. For vitreous cells, a cell count value of 0.5+ indicates 1-10 cells; a cell count value of 1+ indicates 11-20 cells; a cell count value of 2+ indicates 21-30 cells; a cell count value of 3+ indicates 31-100 cells; and a cell count value of 4+ indicates >100 cells. Rare cells were captured as 0.5+ for the analysis shown in this figure.

[0033] FIGS. 7A-7B show optical coherence tomography (OCT) images and retinal thickness maps derived from OCT images taken from subjects 1-6 in Cohort 1 of the study described in Example 1 at a median follow up time of 34 weeks. In addition, the change in BCVA from Baseline, the number of anti-VEGF IVT injections in the 8 months prior to administration of AAV2.7m8-aflibercept, and the number of administered rescue anti-VEGF IVT injections during the study are also provided for each of subjects 1-6. The actual week during which the OCT images and retinal thickness maps were obtained for each subject are indicated (Subject 1 = Week 44; Subject 2 = Week 40; Subject 3 = Week 36; Subject 4 = Week 32; Subject 5 = Week 28; and Subject 6 = Week 28). No subjects required rescue anti-VEGF IVT injections during the study and no retreatment criteria were met at any point during the follow-up period of up to 44 weeks. No subjects exhibited signs of disease re-activation on OCT imaging.

DETAILED DESCRIPTION

[0034] Several aspects are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the features described herein. One having ordinary skill in the relevant art, however, will readily recognize that the features described herein can be practiced without one or more of the specific details or with other methods. The features described herein are not limited by the illustrated ordering of acts or events, as some acts can occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the features described herein.

Definitions

[0035] Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

[0036] The terminology used herein is for the purpose of describing particular examples only and is not intended to be limiting. As used herein, the singular forms “a”, “an” and “the” are intended to include the

plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including”, “includes”, “having”, “has”, “with”, or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising”. The term “comprising” as used herein is synonymous with “including” or “containing”, and is inclusive or open-ended.

[0037] Any reference to “or” herein is intended to encompass “and/or” unless otherwise stated. As used herein, the term “about” a number refers to that number plus or minus 10% of that number. The term “about” a range refers to that range minus 10% of its lowest value and plus 10% of its greatest value.

[0038] The term “subject”, “patient”, or “individual” refers to primates, such as humans and non-human primates, e.g., African green monkeys and rhesus monkeys. In some embodiments, the subject is a human.

[0039] The terms “treat,” “treating”, “treatment,” “ameliorate” or “ameliorating” and other grammatical equivalents as used herein, refer to alleviating, abating or ameliorating an ocular neovascular disease or disorder or symptoms of the ocular neovascular disease or disorder, preventing additional symptoms of the ocular neovascular disease or disorder, ameliorating or preventing the underlying metabolic causes of symptoms, inhibiting the ocular neovascular disease or disorder, e.g., arresting the development of the ocular neovascular disease or disorder, relieving the ocular neovascular disease or disorder, causing regression of the ocular neovascular disease or disorder, or stopping the symptoms of the ocular neovascular disease or disorder, and are intended to include prophylaxis. The terms further include achieving a therapeutic benefit and/or a prophylactic benefit. The term “therapeutic benefit” refers to eradication or amelioration of the ocular neovascular disease or disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the ocular neovascular disease or disorder such that an improvement is observed in the subject, notwithstanding that, in some embodiments, the subject is still afflicted with the ocular neovascular disease or disorder. For prophylactic benefit, the pharmaceutical compositions are administered to a subject at risk of developing the ocular neovascular disease or disorder, or to a subject reporting one or more of the physiological symptoms of the ocular neovascular disease or disorder, even if a diagnosis of the disease or disorder has not been made.

[0040] The terms “administer,” “administering”, “administration,” and the like, as used herein, can refer to the methods that are used to enable delivery of therapeutics or pharmaceutical compositions to the desired site of biological action. These methods include intravitreal or subretinal injection to an eye.

[0041] The terms “effective amount”, “therapeutically effective amount” or “pharmaceutically effective amount” as used herein, can refer to a sufficient amount of at least one pharmaceutical composition or compound being administered which will relieve to some extent one or more of the symptoms of the ocular disease or disorder being treated. An “effective amount”, “therapeutically effective amount” or “pharmaceutically effective amount” of a pharmaceutical composition may be administered to a subject in need thereof as a unit dose (as described in further detail elsewhere herein).

[0042] The term “pharmaceutically acceptable” as used herein, can refer to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of a compound disclosed herein, and is relatively nontoxic (i.e., when the material is administered to an individual it does not cause undesirable biological effects nor does it interact in a deleterious manner with any of the components of the composition in which it is contained).

[0043] The term “pharmaceutical composition,” or simply “composition” as used herein, can refer to a biologically active compound, optionally mixed with at least one pharmaceutically acceptable chemical component, such as, though not limited to carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, excipients and the like.

[0044] An “AAV vector” or “rAAV vector” as used herein refers to an adeno-associated virus (AAV) vector or a recombinant AAV (rAAV) vector comprising a polynucleotide sequence not of AAV origin (e.g., a polynucleotide heterologous to AAV such as a nucleic acid sequence that encodes a therapeutic transgene, e.g., aflibercept) for transduction into a target cell or to a target tissue. In general, the heterologous polynucleotide is flanked by at least one, and generally by two, AAV inverted terminal repeat sequences (ITRs). The term rAAV vector encompasses both rAAV vector particles and rAAV vector plasmids. A rAAV vector may be either single-stranded (ssAAV) or self-complementary (scAAV).

[0045] An “AAV virus” or “AAV viral particle” or “rAAV vector particle” or “rAAV particle” refers to a viral particle comprising at least one AAV capsid protein and a polynucleotide rAAV vector. In some cases, the at least one AAV capsid protein is from a wild type AAV or is a variant AAV capsid protein (e.g., an AAV capsid protein with an insertion, e.g., an insertion of the 7m8 amino sequence as set forth below). If the particle comprises a heterologous polynucleotide (e.g., a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a target cell or target tissue), it is referred to as a “rAAV particle”, “rAAV vector particle” or a “rAAV vector”. Thus, production of rAAV particle necessarily includes production of a rAAV vector, as such a vector contained within a rAAV particle.

[0046] The term “packaging” as used herein can refer to a series of intracellular events that can result in the assembly and encapsidation of a rAAV particle.

[0047] AAV “rep” and “cap” genes refer to polynucleotide sequences encoding replication and encapsidation proteins of adeno-associated virus. AAV rep and cap are referred to herein as AAV “packaging genes.”

[0048] The term “polypeptide” can encompass both naturally occurring and non-naturally occurring proteins (e.g., a fusion protein), peptides, fragments, mutants, derivatives and analogs thereof. A polypeptide may be monomeric, dimeric, trimeric, or polymeric. Further, a polypeptide may comprise a number of different domains each of which has one or more distinct activities. For the avoidance of doubt, a “polypeptide” may be any length greater two amino acids.

[0049] As used herein, “polypeptide variant” or simply “variant” refers to a polypeptide whose sequence contains an amino acid modification. In some embodiments, the modification is an insertion, duplication, deletion, rearrangement or substitution of one or more amino acids compared to the amino acid sequence

of a reference protein or polypeptide, such as a native or wild type protein. A variant may have one or more amino acid point substitutions, in which a single amino acid at a position has been changed to another amino acid, one or more insertions and/or deletions, in which one or more amino acids are inserted or deleted, respectively, in the sequence of the reference protein, and/or truncations of the amino acid sequence at either or both the amino or carboxy termini. A variant can have the same or a different biological activity compared to the reference protein, or the unmodified protein.

[0050] In some embodiments, a variant can have, for example, at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% overall sequence homology to its counterpart reference protein. In some embodiments, a variant can have at least about 90% overall sequence homology to the wild-type protein. In some embodiments, a variant exhibits at least about 95%, at least about 98%, at least about 99%, at least about 99.5%, or at least about 99.9% overall sequence identity.

[0051] As used herein, “recombinant” can refer to a biomolecule, e.g., a gene or protein, that (1) has been removed from its naturally occurring environment, (2) is not associated with all or a portion of a polynucleotide in which the gene is found in nature, (3) is operatively linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature. The term “recombinant” can be used in reference to cloned DNA isolates, chemically synthesized polynucleotide analogs, or polynucleotide analogs that are biologically synthesized by heterologous systems, as well as proteins and/or mRNAs encoded by such nucleic acids. Thus, for example, a protein synthesized by a microorganism is recombinant, for example, if it is synthesized from an mRNA synthesized from a recombinant gene present in the cell.

[0052] The term “anti-VEGF agent” includes any therapeutic agent, including proteins, polypeptides, peptides, fusion protein, multimeric proteins, gene products, antibody, human monoclonal antibody, antibody fragment, aptamer, small molecule, kinase inhibitor, receptor or receptor fragment, or nucleic acid molecule, that can reduce, interfere with, disrupt, block and/or inhibit the activity or function of an endogenous VEGF and/or an endogenous VEGF receptor (VEGFR), or the VEGF-VEGFR interaction or pathway in vivo. An anti-VEGF agent can be any one of the known therapeutic agents that can reduce new blood vessel growth or formation and/or oedem, or swelling, when delivered into a cell, tissue, or a subject in vivo, e.g., ranibizumab, brolizumab, or bevacizumab. In some embodiments, an anti-VEGF agent can be naturally occurring, non-naturally occurring, or synthetic. In some embodiments, an anti-VEGF agent can be derived from a naturally occurring molecule that was subsequently modified or mutated to confer an anti- VEGF activity. In some embodiments, an anti-VEGF agent is a fusion or chimeric protein. In such proteins, functional domains or polypeptides are artificially fused to a moiety or a polypeptide to make a fusion or chimeric protein that can sequester VEGF in vivo or function as a VEGFR decoy. In some embodiments, an anti-VEGF agent is a fusion or chimeric protein that blocks endogenous VEGFR from interacting with its ligands.

[0053] As used herein, “VEGF” can refer to any isoform of VEGF, unless required otherwise, including, but not limited to, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, or any combination, or any functional fragment or variant thereof. Unless required otherwise, “VEGF” can refer

to any member of the VEGF family, including members: VEGF-A, placenta growth factor (PGF), VEGF-B, VEGF-C, and VEGF-D, or any combination, functional fragment, or variant thereof. As used herein, “VEGF receptor” or “VEGFR” or “VEGF-R” can be used to refer to any one of the receptors of VEGF, including, but not limited to, VEGFR-1 (or Flt-1), VEGFR-2 (or Flk-1/KDR), and VEGFR-3 (or Flt-4). VEGFR can be a membrane bound or soluble form, or a functional fragment or truncation of a receptor. Examples of anti-VEGF agent include, but are not limited to, ranibizumab, bevacizumab, brolucizumab, or any combination, variant, or functional fragment thereof.

[0054] “Operatively linked” or “operably linked” or “coupled” can refer to a juxtaposition of genetic elements, wherein the elements are in a relationship permitting them to operate in an expected manner. For instance, a promoter can be operatively linked to a coding region if the promoter helps initiate transcription of the coding sequence. There may be intervening residues between the promoter and coding region so long as this functional relationship is maintained.

[0055] The term “expression vector” or “expression construct” or “cassette” or “plasmid” or simply “vector” can include any type of genetic construct, including AAV or rAAV vectors, containing a nucleic acid or polynucleotide coding for a gene product in which part or all of the nucleic acid encoding sequence is capable of being transcribed and is adapted for gene therapy. The transcript can be translated into a protein. In some embodiments, the transcript is partially translated or not translated. In certain aspects, expression includes both transcription of a gene and translation of mRNA into a gene product. In other aspects, expression only includes transcription of the nucleic acid encoding genes of interest. An expression vector can also comprise control elements operatively linked to the encoding region to facilitate expression of the protein in target cells. The combination of control elements and a gene or genes to which they are operably linked for expression can sometimes be referred to as an “expression cassette,” a large number of which are known and available in the art or can be readily constructed from components that are available in the art.

[0056] The term “heterologous” can refer to an entity that is genotypically distinct from that of the rest of the entity to which it is being compared. For example, a polynucleotide introduced by genetic engineering techniques into a plasmid or vector derived from a different species can be a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence with which it is not naturally found linked can be a heterologous promoter.

[0057] As used herein, “7m8” refers to the amino acid sequence LALGETTRPA (SEQ ID NO: 1).

[0058] “7m8 variant” refers to a rAAV, which can be of any serotype, with the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted in the solvent exposed GH loop of the capsid protein.

[0059] When 7m8 is inserted in a rAAV2 (also referred to as AAV2.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 570-611 of the AAV2 capsid protein, e.g., between positions 587 and 588 of the AAV2 capsid protein, VP1. In some cases, when 7m8 is inserted in a rAAV2 (also referred to as AAV2.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop of the AAV2 capsid protein, e.g., between

positions 587 and 588 of AAV2 VP1 comprising the sequence of SEQ ID NO: 13. When 7m8 is inserted in a rAAV1 (also referred to as AAV1.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 571-612 of AAV1 of the AAV1 capsid protein, e.g., between amino acids 590 and 591 of the AAV1 capsid protein. When 7m8 is inserted in a rAAV5 (also referred to as AAV5.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 560-601 of AAV5 of the AAV5 capsid protein, e.g., between amino acids 575 and 576 of the AAV5 capsid protein. When 7m8 is inserted in a rAAV6 (also referred to as AAV6.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 571 to 612 of the AAV6 capsid protein, e.g., between amino acids 590 and 591 of the AAV6 capsid protein. When 7m8 is inserted in a rAAV7 (also referred to as AAV7.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 572 to 613 of the AAV7 capsid protein, e.g., between amino acids 589 and 590 of the AAV7 capsid protein. When 7m8 is inserted in a rAAV8 (also referred to as AAV8.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 573 to 614 of the AAV8 capsid protein, e.g., between amino acids 590 and 591 of the AAV8 capsid protein. When 7m8 is inserted in a rAAV9 (also referred to as AAV9.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop of the AAV9 capsid protein, e.g., between amino acids 588 and 589 of the AAV9 capsid protein. When 7m8 is inserted in a rAAV10 (also referred to as AAV10.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop within amino acids 573 to 614 of the AAV10 capsid protein, e.g., between amino acids 589 and 590 of the AAV10 capsid protein.

Overview

[0060] Current therapies (e.g., aflibercept) for ocular neovascular diseases such as wAMD require lifelong IVT administration approximately every 4-8 weeks. This can increase the risk of inflammation, infection, and other adverse effects in some patients. Further, current therapies create compliance challenges due to repeated and/or frequent trips to medical offices for administration of the therapy, especially in elderly patients, who are most affected with wAMD. Reduction in frequency of administration is associated with vision loss and deterioration of the eye disease or condition. The ability of AAV vectors to efficiently transduce target retinal cells following IVT injection has been exploited to successfully transfer therapeutic genes into photoreceptors, retinal pigment epithelium, and the inner retina to treat a variety of retinal diseases. Thus, administration of rAAV particles encoding an anti-VEGF agent (e.g., aflibercept) can provide prolonged and/or sustained release of the anti-VEGF agent *in vivo*.

[0061] Surprisingly, administration of a single low unit dose of 6×10^{11} vector genomes (vg) per eye of rAAV particles encoding aflibercept to the eyes of individuals with an ocular neovascular disease led to stabilization of the disease and a robust anatomical response in all treated individuals (*See Example 1*). In addition, visual acuity was stabilized in all treated individuals and none of the individuals required rescue anti-VEGF treatment (e.g., aflibercept IVT injections) after administration of the single low unit dose of 6

$\times 10^{11}$ vg/eye of rAAV particles encoding aflibercept. Moreover, administration of the single unit dose of rAAV particles encoding aflibercept to the eyes of individuals with an ocular neovascular disease unexpectedly caused a reduction (e.g., resolution) of symptoms, including intraretinal and subretinal fluid that were refractory to prior anti-VEGF treatments (e.g., chronic IVT injections of aflibercept, ranibizumab, or bevacizumab).

[0062] Accordingly, the present disclosure provides methods of treating an ocular neovascular disease in an individual by administering a single unit dose of 6×10^{11} vg/eye or less of rAAV particles encoding an anti-VEGF agent (e.g., aflibercept). In addition, the present disclosure provides methods for reducing retinal fluid in the eye of an individual with an ocular neovascular disease by administering a single unit dose of rAAV particles encoding an anti-VEGF agent (e.g., aflibercept). The methods disclosed herein reduce or eliminate the need for repeated IVT injections while providing long-term efficacy, thereby addressing the non-compliance and non-adherence problem. In addition, the methods provided herein reduce the adverse effects associated with multiple IVT injections.

Methods of Treatment

[0063] Provided herein is a method for treating an ocular neovascular disease in an individual, the method comprising administering a unit dose of recombinant adeno-associated virus (rAAV) particles to an eye of the individual.

[0064] Also provided herein is a method for reducing retinal fluid in the eye of an individual with an ocular neovascular disease, the method comprising administering a unit dose of rAAV particles to an eye of the individual.

[0065] In some embodiments, the ocular neovascular disease is wet age-related macular degeneration (wAMD), 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.

[0066] In some embodiments, the individual is a human. In some embodiments, the individual received at least one prior treatment (e.g., at least one, at least two, at least three, at least four, at least 5 or more treatments) for the ocular neovascular disease with an anti-VEGF agent (e.g., bevacizumab, brotacizumab, ranibizumab, faricimab, abicipar pegol, and/or aflibercept) in about the last 8 weeks, about the last 9 weeks, about the last 10 weeks, about the last 11 weeks, about the last 12 weeks, about the last 13 weeks, about the last 14 weeks, about the last 15 weeks, or about the last 16 weeks prior to administration of the unit dose of rAAV particles. In some embodiments, the individual demonstrated a meaningful response to a prior treatment with anti-VEGF agent (e.g., bevacizumab, brotacizumab, ranibizumab, faricimab, abicipar pegol, and/or aflibercept). In some embodiments, the anti-VEGF agent is aflibercept, a functional variant thereof, or a functional fragment thereof. In some embodiments, the anti-VEGF agent comprises a polypeptide comprising an amino acid sequence with at least about 95% identity

to the amino acid sequence of SEQ ID NO: 35. In some embodiments, the retinal fluid in the eye of an individual is intraretinal fluid (IRF) and/or subretinal fluid (SRF). In some embodiments, the amount or presence of retinal fluid in the eye of the individual is refractory to prior treatment with an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, and/or aflibercept). In some embodiments, the anti-VEGF agent is aflibercept, a functional variant thereof, or a functional fragment thereof. In some embodiments, the anti-VEGF agent comprises a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35.

[0067] In some embodiments, the unit dose is expressed as the number of vector genomes (vg). In some embodiments, the unit dose is about 6×10^{11} vector genomes (vg) or less of the rAAV particles. In some embodiments, the unit dose is expressed as the number of vector genomes (vg) per eye (vg/eye). In some embodiments, the unit dose is about 6×10^{11} vg/eye or less of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} to about 2×10^{11} vg/eye. In some embodiments, the unit dose of rAAV particles is about 2×10^{11} or about 6×10^{10} vg/eye.

[0068] In some embodiments, the unit dose of rAAV particles is administered to one eye of the individual. In some embodiments, the one eye of the individual is the right eye or the left eye. In some embodiments, the one eye of the individual is the right eye. In some embodiments, the one eye of the individual is the left eye. In some embodiments, the methods provided herein further comprise administering a unit dose of rAAV particles to the contralateral eye of the individual. In some embodiments, the one eye of the individual is the right eye and the contralateral eye is the left eye. In some embodiments, the one eye of the individual is the left eye and the contralateral eye is the right eye.

[0069] In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye of the individual is up to about 2 weeks (e.g., about 0 days, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days) after administering the unit dose of rAAV particles to the one eye. In some embodiments, the unit dose of rAAV particles administered to the contralateral eye of the individual is about the same (e.g., less than 1% higher or lower, less than 5% higher or lower, less than 10% higher or lower, or less than 20% higher or lower) or lower (e.g., about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% lower) than the unit dose of rAAV particles administered to the one eye of the individual.

[0070] In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye is at least about 2 weeks (e.g., at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 1 year, at least about 2 years, at least about 3 years, at least about 4 years, at least about 5 years, or more) after administering the unit dose of rAAV particles to the one eye. In some embodiments, the unit dose of rAAV particles administered to the contralateral eye of the individual is higher (e.g., more than any of about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about

80%, about 90%, about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300% or more) than the unit dose of rAAV particles administered to the one eye of the individual.

[0071] In some embodiments, the rAAV particles comprise a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.99%, or 100% 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. The sequence of SEQ ID NO: 35 is provided below:

SDTGRPFVEMYSEIPEIIHMTGRELVI PCRVTSPNITVTLLKKFPLDTLIPDGKRIIWDSRKGFII SNATY
KEIGLLTCEATVNGHLYKTNLYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELVNGIDFNWEYPS
SKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPE
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSL
SLSPG (SEQ ID NO: 35)

[0072] In some embodiments, 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.

[0073] In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.99%, or 100% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise 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). In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 35. In some embodiments, the polypeptide is aflibercept or a functional variant thereof or functional fragment thereof.

[0074] In some embodiments, the rAAV particles comprise a nucleic acid comprising a codon-optimized sequence encoding an amino acid sequence with at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about

99%, at least about 99.99%, or 100% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid comprising a codon-optimized sequence encoding 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). In some embodiments, the rAAV particles comprise a nucleic acid comprising a codon-optimized sequence encoding an amino acid sequence with 100% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs).

[0075] In some embodiments, the rAAV particles comprise a nucleic acid comprising the cDNA sequence of aflibercept or a functional variant thereof or functional fragment thereof and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid comprising a codon-optimized cDNA sequence of aflibercept or a functional variant thereof or functional fragment thereof and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid comprising the nucleic acid sequence of SEQ ID NO: 36.

[0076] In some embodiments, the nucleic acid further comprises (a) a first enhancer region comprising a CMV sequence; (b) a promoter region comprising a CMV sequence; (c) a 5'UTR region comprising, in the 5' to 3' order, a TPL sequence and an eMLP sequence; (d) a second enhancer region comprising a full EES sequence; and (e) a HGH polyadenylation site. In some embodiments, the enhancer region comprising a CMV sequence comprises the sequence of SEQ ID NO: 22. In some embodiments, the promoter region comprising a CMV sequence comprises the sequence of SEQ ID NO: 23. In some embodiments, the TPL sequence comprises the sequence of SEQ ID NO: 24. In some embodiments, the eMLP sequence comprises the sequence of SEQ ID NO: 25. In some embodiments, the second enhancer region comprising a full EES sequence comprises the sequence of SEQ ID NO: 26. In some embodiments, the HGH polyadenylation site comprises the sequence of SEQ ID NO: 27.

[0077] In some embodiments, the rAAV particles comprise an AAV2 capsid protein comprising 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. The sequence of SEQ ID NO: 13 is provided below:

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MAADGYLPDWLEDTLSEGIQWWKLKPGPPPKPAERHKDDSRGLVLPGYKYLGPFNGLD
KGEFVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
AKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDAD
SVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVI
TTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDNRFHCHFSPRDWQRLI
NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLSTVQVFTDSEYQLPYVLGSAHQG
CLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF
HSSYAHSQSLDRLMNPLIDQYLYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPG
PCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVPNPGPAMASHKDDEEKFFPQSGVL
IFGKQGSEKTNVDIEKVMITDEEEIIRTTPVATEQYGSVSTNLQRGNRQAATADVNTQGV
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LPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPQILIKNTPVPANPSTT
 FSAAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNKNKSVNVDFTVDTNGVY
 SEPRPIGTRYLTRNL (SEQ ID NO: 13)

[0078] In some embodiments, the rAAV particles comprise an AAV2 capsid protein comprising 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 embodiments, the rAAV particles comprise an AAV2 capsid protein comprising 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.

[0079] In some embodiments, the rAAV particles comprise an AAV2 capsid protein comprising any of the following amino acid sequences inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein: LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8), LAKDTDTTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), LASTGKVPNA (SEQ ID NO: 12), LGETTRP (SEQ ID NO: 14), NETITRP (SEQ ID NO: 15), KAGQANN (SEQ ID NO: 16), KDPKTTN (SEQ ID NO: 17), KDTDTTR (SEQ ID NO: 18), RAGGSVG (SEQ ID NO: 19), AVDTTKF (SEQ ID NO: 20), and STGKVPN (SEQ ID NO: 21). In some embodiments, the rAAV particles comprise an AAV2 capsid protein comprising any of the following amino acid sequences inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13: LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8), LAKDTDTTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), LASTGKVPNA (SEQ ID NO: 12), LGETTRP (SEQ ID NO: 14), NETITRP (SEQ ID NO: 15), KAGQANN (SEQ ID NO: 16), KDPKTTN (SEQ ID NO: 17), KDTDTTR (SEQ ID NO: 18), RAGGSVG (SEQ ID NO: 19), AVDTTKF (SEQ ID NO: 20), and STGKVPN (SEQ ID NO: 21).

[0080] In some embodiments, the administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual is by intravitreal (IVT) injection, intraocular administration, or intraretinal injection. In some embodiments, the administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual is by intravitreal (IVT) injection.

[0081] In some embodiments, the unit dose of rAAV particles is in a pharmaceutical formulation. In some embodiments, the pharmaceutical formulation comprises the rAAV particles, one or more osmotic or ionic strength agents, one or more buffering agents, one or more surfactants, and one or more solvents. In some embodiments, the osmotic or ionic strength agent is sodium chloride. In some embodiments, the one or more buffering agents are sodium phosphate monobasic and/or sodium phosphate dibasic. In some

embodiments, the surfactant is Poloxamer 188. In some embodiments, the solvent is water. In some embodiments, the pharmaceutical formulation comprises the rAAV particles, sodium chloride, sodium phosphate and a surfactant. In some embodiments, the pharmaceutical formulation comprises about 1×10^{10} vg/mL to about 1×10^{13} vg/mL of rAAV particles. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL to about 6×10^{12} vg/mL of rAAV particles. In some embodiments, the pharmaceutical formulation comprises about 150 mM to about 200 mM sodium chloride (e.g., any of about 150 mM, about 160 mM, about 170 mM, about 180 mM, about 190 mM, or about 200 mM). In some embodiments, the pharmaceutical formulation comprises about 1 mM to about 10 mM monobasic sodium phosphate (e.g., about 1mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, or about 10 mM). In some embodiments, the pharmaceutical formulation comprises about 1 mM to about 10 mM dibasic sodium phosphate (e.g., about 1mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, or about 10 mM). In some embodiments, the pharmaceutical formulation comprises about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188 (e.g., any of about 0.0005% (w/v), 0.0006% (w/v), 0.0007% (w/v), 0.0008% (w/v), 0.0009% (w/v), 0.001% (w/v), 0.002% (w/v), 0.003% (w/v), 0.004% (w/v), or about 0.005% (w/v)). In some embodiments, the pharmaceutical formulation has a pH of about 7.0 to about 7.5 (e.g., any of about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, or about 7.5). In some embodiments, the pharmaceutical formulation comprises about 6×10^{12} vg/mL of rAAV particles, about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL of rAAV particles, about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3.

[0082] In some embodiments, the unit dose of rAAV particles comprises a volume of between about 25 μ L to about 250 μ L (e.g., any of about 25 μ L, about 30 μ L, about 40 μ L, about 50 μ L, about 60 μ L, about 70 μ L, about 80 μ L, about 90 μ L, about 100 μ L, about 110 μ L, about 120 μ L, about 130 μ L, about 140 μ L, about 150 μ L, about 160 μ L, about 170 μ L, about 180 μ L, about 190 μ L, about 200 μ L, about 210 μ L, about 220 μ L, about 230 μ L, about 240 μ L, or about 250 μ L). In some embodiments, the concentration of rAAV particles in the pharmaceutical formulation is adjusted such that the volume of the unit dose of rAAV particles administered to an eye of the individual is between about 25 μ L to about 250 μ L. In some embodiments, the unit dose of rAAV particles comprises a volume of about 100 μ L. In some embodiments, the unit dose of rAAV particles comprises a volume of about 30 μ L.

[0083] In some embodiments, the unit dose of rAAV particles is administered in combination with steroid treatment. In some embodiments, the steroid treatment is a corticosteroid treatment. In some embodiments, the steroid treatment is a systemic steroid treatment. In some embodiments, the steroid treatment is an oral steroid treatment. In some embodiments, the steroid treatment is a prednisone

treatment. In some embodiments, the steroid treatment is an ophthalmic steroid treatment. In some embodiments, the ophthalmic steroid treatment is a topical steroid treatment (e.g. a drop), a periocular steroid treatment (e.g. subtenons, subconjunctival), an intravitreal steroid treatment, or a superchoroidal steroid treatment. In some embodiments, the topical steroid treatment is a difluprednate treatment, a medrysone treatment, a loteprednol treatment, a prednisolone treatment, a fluocinolone treatment, a triamcinolone treatment, a rimexolone treatment, a dexamethasone treatment, a fluorometholone treatment, a fluocinolone treatment, a rimexolone treatment, or a prednisone treatment. In some embodiments, the topical steroid treatment is a difluprednate treatment. In some embodiments, the steroid treatment is administered before, during, and/or after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered during administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before and during administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before and after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered during, and after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before, during, and after administration of the unit dose of rAAV particles.

[0084] In some embodiments, the steroid treatment is an ophthalmic steroid treatment (e.g., difluprednate). In some embodiments, the ophthalmic steroid treatment (e.g., difluprednate) is a daily steroid treatment for up to about 4 weeks, about 6 weeks, or about 8 weeks from administering the unit dose of rAAV particles. In some embodiments, the ophthalmic steroid treatment comprises about four administrations of ophthalmic steroid on about week 1, about three administrations of ophthalmic steroid on about week 2, about two administrations of ophthalmic steroid on about week 3, and about one administration of ophthalmic steroid on about week 4; timing starting with and following administration of the unit dose of rAAV particles. In some embodiments, the ophthalmic steroid is about 0.005% to about 0.5% difluprednate. In some embodiments, the ophthalmic steroid is any of about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.4%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, or about 0.1% difluprednate. In some embodiments, the ophthalmic steroid is difluprednate 0.05%. In some embodiments, a dose of difluprednate 0.05% is one drop of ophthalmic solution. In some embodiments, one drop is about 50 μ l (e.g., about 25 μ l to about 50 μ l, about 50 μ l to about 100 μ l). In some embodiments, a dose of difluprednate comprises about 1 μ g to about 5 μ g, or about 2 μ g to about 3 μ g, or about 2.5 μ g difluprednate. In some embodiments, a dose of difluprednate comprises about 2.5 μ g difluprednate.

[0085] In some embodiments, the steroid treatment is an ophthalmic steroid treatment (e.g., difluprednate). In some embodiments, the ophthalmic steroid treatment (e.g., difluprednate) is a daily topical steroid treatment for up to about 4 weeks, about 6 weeks, or about 8 weeks from administering the

unit dose of rAAV particles. In some embodiments, 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 embodiments, the topical steroid comprises difluprednate 0.05% at a dose of about 1 µg to about 3 µg. In some embodiments, the topical steroid comprises difluprednate 0.05% at a dose of about 2.5 µg. In some embodiments, the topical steroid is about 0.005% to about 0.5% difluprednate. In some embodiments, the topical steroid is any of about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.4%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, or about 0.1% difluprednate. In some embodiments, the topical steroid is difluprednate 0.05%. In some embodiments, a dose of difluprednate 0.05% is one drop of ophthalmic solution. In some embodiments, one drop is about 50 µl (e.g., about 25 µl to about 50 µl, about 50 µl to about 100 µl). In some embodiments, a dose of difluprednate comprises about 1 µg to about 5 µg, or about 2 µg to about 3 µg, or about 2.5 µg difluprednate. In some embodiments, a dose of difluprednate comprises about 2.5 µg difluprednate.

[0086] In some embodiments, the retinal fluid in the eye of the individual (e.g., SRF and/or IRF) is reduced by more than any of about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 100% after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral of the individual. In some embodiments, the retinal fluid in the eye of the individual (e.g., SRF and/or IRF) is reduced by more than any of about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 100% after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral of the individual compared to the level of retinal fluid in the eye of the individual prior to administration of the unit dose or rAAV particles. In some embodiments, the retinal fluid in the eye of the individual (e.g., SRF and/or IRF) is reduced by about 100% after administration of the unit dose of rAAV particles to the one eye and/or to the contralateral of the individual compared to the level of retinal fluid in the eye of the individual prior to administration of the unit dose or rAAV particles.

[0087] In some embodiments, the methods provided herein further comprising monitoring the level of retinal fluid (e.g., SRF and/or IRF) in the one eye and/or the contralateral eye of the individual after administration of the unit dose of rAAV particles. In some embodiments, the reduction of retinal fluid (e.g., SRF and/or IRF) in the eye is first observed any of about 1 day, about 3 days, about 8 days, about 2 weeks, about 4 weeks, about 6 weeks, about 8 weeks, about 12 weeks, about 16 weeks, about 20 weeks, about 24 weeks, about 28 weeks, about 32 weeks, about 36 weeks, about 40 weeks, about 44 weeks, about 48 weeks, about 52 weeks, about 56 weeks, about 60 weeks, about 64 weeks, about 68 weeks, about 72 weeks, about 76 weeks, about 80 weeks, about 84 weeks, about 88 weeks, about 92 weeks, about 96 weeks, about 100 weeks, about 104 weeks, or more after administration of the unit dose of rAAV particles. In some embodiments, the reduction of retinal fluid (e.g., SRF and/or IRF) in the eye continues

or is maintained for at least 1 week, at least 2 weeks, at least 4 weeks, at least 6 weeks, at least 8 weeks, at least 12 weeks, at least 16 weeks, at least 20 weeks, at least 24 weeks, at least 28 weeks, at least 32 weeks, at least 36 weeks, at least 40 weeks, at least 44 weeks, at least 48 weeks, at least 52 weeks, at least 56 weeks, at least 60 weeks, at least 64 weeks, at least 68 weeks, at least 72 weeks, at least 76 weeks, at least 80 weeks, at least 84 weeks, at least 88 weeks, at least 92 weeks, at least 96 weeks, at least 100 weeks, at least 104 weeks, or more after administration of the unit dose of rAAV particles.

[0088] In some embodiments, the reduction of retinal fluid (e.g., SRF and/or IRF) in the eye is determined by any method known in the art. In some embodiments, the reduction of retinal fluid (e.g., SRF and/or IRF) in the eye is determined by optical coherence tomography (OCT), spectral domain OCT (SD-OCT), OCT angiography, fluorescein angiography, or by direct retinal observation. In some embodiments, the reduction of retinal fluid (e.g., SRF and/or IRF) in the eye is determined by optical coherence tomography (OCT). In some embodiments, the reduction of retinal fluid (e.g., SRF and/or IRF) in the eye is determined by spectral domain OCT (SD-OCT). In some embodiments, the reduction of retinal fluid (e.g., SRF and/or IRF) in the eye is determined by OCT angiography. In some embodiments, the reduction of retinal fluid (e.g., SRF and/or IRF) in the eye is determined by fluorescein angiography. In some embodiments, the reduction of retinal fluid (e.g., SRF and/or IRF) in the eye is determined by direct retinal observation.

[0089] In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye is assessed based on the level of retinal fluid (e.g., intraretinal fluid (IRF) and/or subretinal fluid (SRF)) compared the level of retinal fluid (e.g., SRF and/or IRF) prior to administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye (e.g., as described above). In some embodiments, the retinal fluid is subretinal fluid (SRF) or intraretinal fluid (IRF). In some embodiments, the retinal fluid is subretinal fluid (SRF). In some embodiments, the retinal fluid is intraretinal fluid (IRF). In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye is determined if a reduction in retinal fluid (e.g., IRF and/or SRF) is observed after administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye compared to the levels of retinal fluid (e.g., IRF and/or SRF) prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye (e.g., as described above). In some embodiments, the ocular neovascular disease is wAMD.

[0090] In some embodiments, 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 embodiments, 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 of retinal thickness compared to the retinal thickness prior to administration of the unit dose of rAAV particles. In some embodiments, retinal thickness is central subfield thickness (CST) or central retinal thickness (CRT). In some embodiments, 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 of retinal thickness of more than any of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or about 100% compared to the retinal thickness prior to administration of the unit dose of rAAV particles. In some embodiments, the retinal thickness (e.g., CST or CRT) is determined by OCT or SD-OCT. In some embodiments, 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 of central retinal thickness (CRT) or central subfield thickness (CST) of about 10 μm to about 100 μm (e.g., more than any of about 10 μm , about 15 μm , about 20 μm , about 25 μm , about 30 μm , about 35 μm , about 40 μm , about 45 μm , about 50 μm , about 55 μm , about 60 μm , about 65 μm , about 70 μm , about 75 μm , about 80 μm , about 85 μm , about 90 μm , about 95 μm , about 100 μm , or more). In some embodiments, 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 of central retinal thickness (CRT) or central subfield thickness (CST) of more than any of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or about 100% compared to the retinal thickness prior to administration of the unit dose of rAAV particles. In some embodiments, 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 of central retinal thickness (CRT) or central subfield thickness (CST) of at least about 10% compared to the retinal thickness prior to administration of the unit dose of rAAV particles. In some embodiments, 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 of central retinal thickness (CRT) or central subfield thickness (CST) of about 15% or more compared to the retinal thickness prior to administration of the unit dose of rAAV particles.

[0091] In some embodiments, 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 embodiments, 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 embodiments, 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 of more than any of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50% compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, 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 of at least about 10% compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, the macular volume is determined by OCT or SD-OCT. In

some embodiments, 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 of at least about 10% compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, 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 of about 15% or more compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, the macular volume is determined by OCT or SD-OCT.

[0092] In some embodiments, 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 embodiments, 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 embodiments, 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 of more than any of about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300%, or more compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodiments, visual acuity is best corrected visual acuity (BCVA). In some embodiments, 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 BCVA compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, BCVA is expressed as an ETDRS score, which corresponds to the number of letters correctly read (Vitale *et al.*, (2016) JAMA Ophthalmol 134(9):1041:1047). In some embodiments, 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 BCVA of at least 15 ETDRS letters (Vitale *et al.*, (2016) JAMA Ophthalmol 134(9):1041:1047) (e.g., at least about 15, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, or about 70 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles.). In some embodiments, 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 BCVA of about 5 ETDRS letters compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses fewer than 15 ETDRS letters (Vitale *et al.*, (2016) JAMA Ophthalmol 134(9):1041:1047) (e.g., any of 15 or less, 14 or less, 13 or less, 12 or less, 11 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, 2 or less, 1, or 0 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the administering the unit dose of rAAV particles to the one eye and/or to the

contralateral eye of the individual results in maintenance of BCVA, wherein the individual loses about 2 letters compared to the BCVA prior to administration of the unit dose of rAAV particles.

[0093] In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on best corrected visual acuity (BCVA) in the one eye and/or the contralateral eye. In some embodiments, BCVA is expressed as an ETDRS score, which corresponds to the number of letters correctly read (Vitale *et al.*, (2016) JAMA Ophthalmol 134(9):1041:1047). In some embodiments, an individual is determined to have maintenance of vision and/or visual acuity if the individual loses fewer than 15 letters in an ETDRS score (e.g., any of 15 or less, 14 or less, 13 or less, 12 or less, 11 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, 2 or less, 1, or 0 letters) compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, an individual is determined to have an improvement of vision and/or visual acuity if the individual gains at least 15 letters (e.g., any of at least about 15, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, or about 70 letters) compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0094] In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on central subfield thickness (CST) or central retinal thickness (CRT) in the one eye and/or the contralateral eye. In some embodiments, CST or CRT is determined by SD-OCT. In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if the CST or CRT assessed by SD-OCT is decreased after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if the CST or CRT assessed by SD-OCT is maintained after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0095] In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on macular volume in the one eye and/or the contralateral eye. In some embodiments, macular volume is determined by SD-OCT. In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if the macular volume assessed by SD-OCT is decreased after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV

particles in the one eye and/or the contralateral eye is determined if the macular volume assessed by SD-OCT is maintained after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0096] In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on retinal thickness (e.g., central retinal thickness (CRT) or central subfield thickness (CST)) and macular volume in the one eye and/or the contralateral eye. In some embodiments, CST and macular volume are determined by SD-OCT. In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if the CST and macular volume assessed by SD-OCT are decreased after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if the CST and macular volume assessed by SD-OCT are maintained after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0097] In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on the number of rescue therapy treatments (e.g., aflibercept injections) required by the individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if an individual requires less than one rescue therapy treatment (e.g., aflibercept injection) any of every 4 weeks, every 5 weeks, every 6 weeks, every 7 weeks, every 8 weeks, every 9 weeks, every 10 weeks, or more after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0098] In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if an individual does not require any rescue therapy treatment (e.g., aflibercept injection) for any of at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 15 weeks, at least 20 weeks, at least 30 weeks, at least 40 weeks, at least 50 weeks, at least 60 weeks, at least 70 weeks, at least 80 weeks, at least 90 weeks, at least 100 weeks, at least 110 weeks, or more after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0099] In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed

based on the level of retinal fluid compared the level of retinal fluid prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if a reduction in retinal fluid is observed after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to the level of retinal fluid prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the ocular neovascular disease is wAMD.

[0100] In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on the resolution of pigment epithelial detachment (PED) compared to PED prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if resolution of PED after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is observed, compared to PED prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the ocular neovascular disease is wAMD.

[0101] In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on choroidal neovascularization (CNV) lesion growth as determined by fluorescein angiography. In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if CNV lesions shrink (e.g., by more than any of about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or 100%) after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to CNV lesions present prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is determined if CNV lesions do not grow (e.g., grow less than any of about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 15%, or about 20%) after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye compared to CNV lesions present prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the ocular neovascular disease is wAMD.

[0102] In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on the anatomical features of the one eye and/or the contralateral eye based on any methods known in the art (e.g., SD-OCT, OCT, fluorescein angiography, digital color fundus photography, etc.). In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit

dose of rAAV particles in the one eye and/or the contralateral eye is determined if an improvement in anatomical features of the one eye and/or the contralateral eye is observed after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the ocular neovascular disease is wAMD.

[0103] In some embodiments, treatment of an ocular neovascular disease in an individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye is assessed based on ophthalmologic examination, intraocular pressure (e.g., using a Goldmann applanation tonometer or Tono-pen), indirect ophthalmoscopy, examination of the one eye and/or the contralateral eye and adnexa, eyelid and/or pupil responsiveness, belpharoptosis, abnormal pupil shape, unequal pupils, abnormal reaction to light, afferent pupillary defects, slit-lamp examination (including of the eyelids, conjunctiva, cornea, lens, iris, and anterior chamber), posterior segment abnormalities of the vitreous, optic nerve, peripheral retina, and retinal vasculature, SD-OCT, fluorescein angiography, digital color fundus photography (including images of the retina, optic disc, and/or macula), aqueous humor sampling, vitreous humor sampling, OCT-angiography (OCT-A), refraction and/or visual acuity (BCVA). In some embodiments, SD-OCT is performed to evaluate retinal thickness (e.g., central retinal thickness or central subfield thickness), macular volume, and/or the presence of fluid (e.g., subretinal fluid or intraretinal fluid). In some embodiments, the ocular neovascular disease is wAMD.

[0104] The unit dose of rAAV particles may be administered to the one eye and/or to the contralateral eye of the individual by any method known in the art. For example, the unit dose of rAAV particles may be administered to the one eye and/or to the contralateral eye of the individual intraocularly, or by intravitreal injection. In some embodiments, the administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual is intraocular. In some embodiments, the administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual is by intravitreal injection (IVT) or subretinal injection. In some embodiments, the administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual is by IVT injection. In some embodiments, aseptic technique is employed to administer a unit dose of rAAV particles by intravitreal injection. In some embodiments, aseptic technique with providone-iodine is employed to administer a unit dose of rAAV particles by intravitreal injection.

[0105] In some embodiments, the individual has not received a prior treatment for an ocular neovascular disease. In some embodiments, the individual has not received a prior treatment in the one eye and/or the contralateral eye for an ocular neovascular disease. In some embodiments, the individual has not received a prior treatment with an anti-VEGF agent (e.g., bevacizumab, brotacizumab, ranibizumab, faricimab, abicipar pegol, and/or aflibercept). In some embodiments, the individual has not received a prior treatment with an anti-VEGF agent (e.g., bevacizumab, brotacizumab, ranibizumab, faricimab, abicipar pegol, and/or aflibercept) in the one eye and/or the contralateral eye. In some embodiments, the individual has not received a prior aflibercept treatment. In some embodiments, the individual has not received a prior aflibercept treatment in the one eye and/or the contralateral eye.

Steroid Treatments

[0106] In some embodiments, the unit dose of rAAV particles is administered in combination with steroid treatment. In some embodiments, the steroid treatment is a corticosteroid treatment. Exemplary corticosteroids include, without limitation, aclometasone, amcinomide, beclomethasone, betamethasone, budesonide, ciclesonide, clobetasol, clobetasone, clocortolone, cloprednol, cortivazol, deflazacort, deoxycorticosterone, desonide, desoximetasone, dexamethasone, diflorasone, diflucortolone, difluprednate, flucorolone, fludrocortisone, fludroxycortide, flumetasone, flunisolide, fluocinolone acetate, fluocinolone, fluocortin, fluocortolone, fluorometholone, fluperolone, fluticasone, fuprednidene, formocortal, halcinonide, halometasone, hydrocortisone aceponate, hydrocortisone buteprate, hydrocortisone butyrate, loteprednol, medrysone, meprednisone, methylprednisolone, methylprednisolone aceponate, mometasone furoate, paramethasone, prednicarbate, prednisone, prednisolone, prednylidene, remexolone, tixocortol, triamcinolone, and ulobetasol. In some embodiments, the steroid treatment is a systemic steroid treatment. In some embodiments, the steroid treatment is an oral steroid treatment. In some embodiments, the steroid treatment is an ophthalmic steroid treatment. In some embodiments, the ophthalmic steroid treatment is a topical steroid treatment (e.g. a drop), a periocular steroid treatment (e.g. subtenons, subconjunctival), an intravitreal steroid treatment, or a superchoroidal steroid treatment. In some embodiments, the topical steroid treatment is a difluprednate treatment, a medrysone treatment, a loteprednol treatment, a prednisolone treatment, a fluocinolone treatment, a triamcinolone treatment, a rimexolone treatment, a dexamethasone treatment, a fluorometholone treatment, a fluocinolone treatment, a rimexolone treatment, or a prednisone treatment. In some embodiments, the ophthalmic steroid treatment is a difluprednate treatment. In some embodiments, the steroid treatment is a prednisone treatment. In some embodiments, the steroid treatment is a difluprednate treatment.

[0107] In some embodiments, the steroid treatment comprises a systemic steroid treatment and a topical steroid treatment. In some embodiments, the systemic steroid treatment is an oral steroid treatment. In some embodiments, the systemic steroid treatment is a prednisone treatment. In some embodiments, the topical steroid treatment is a difluprednate treatment. In some embodiments, the systemic steroid treatment and the topical steroid treatment are administered simultaneously (e.g., on the same day). In some embodiments, the systemic steroid treatment and the topical steroid treatment are administered separately (e.g., on different days).

[0108] In some embodiments, the steroid is administered before, during, and/or after administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered before, during, and after administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered during, and after administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered before administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered during administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered before and during administration of the unit dose of rAAV particles. In some

embodiments, the steroid is administered after administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered during and after administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered before and/or after administration of the unit dose of rAAV particles. In some embodiments, the steroid is administered before and after administration of the unit dose of rAAV particles.

[0109] In some embodiments, the steroid treatment is a systemic steroid treatment. In some embodiments, the systemic steroid treatment is an oral steroid treatment. In some embodiments, the steroid treatment is an oral prednisone treatment. In some embodiments, the oral prednisone treatment is initiated prior to administration of the unit dose of rAAV particles. In some embodiments, an initial oral prednisone treatment is administered at a dose of any of about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, or about 70 mg of prednisone per day any of about 7 days, about 6 days, about 5 days, about 4 days, about 3 days, about 2 days, about 1 day, or 0 days before administration of the unit dose of rAAV particles, and is continued for any of about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, or about 10 days or more. In some embodiments, an initial oral prednisone treatment is administered at a dose of about 60 mg of prednisone per day about 3 days before administration of the unit dose of rAAV, and is continued for about 3 days.

[0110] In some embodiments, the initial oral prednisone treatment is followed by an oral prednisone treatment dose taper. In some embodiments, the oral prednisone treatment dose taper is administered at a dose of any of about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, or about 50 mg of prednisone per day for a total of any of about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, or about 7 days, followed by a dose of about 10 mg, about 15 mg, about 20 mg or about 25 mg of prednisone per day for any of about 1 day, about 2 days, about 3 days, or about 4 days, followed by a dose of about 5 mg, about 10 mg, or about 15 mg of prednisone per day for about 1 day, about 2 days, about 3 days, or about 4 days. In some embodiments, the prednisone dose taper is administered at a dose of any of about 40 mg of prednisone per day for 3 days, followed by a dose of about 20 mg of prednisone per day for 2 days, followed by a dose of about 10 mg of prednisone per day for 2 days.

[0111] In some embodiments, an initial oral prednisone treatment is initiated 3 days before to administration of the unit dose of rAAV particles at a dose of 60 mg of prednisone per day for a total of 6 days, followed by a dose of 40 mg of prednisone per day for a total of 3 days, followed by a dose of 20 mg of prednisone per day for 2 days, followed by a dose of 10 mg of prednisone per day for 2 days.

[0112] In some embodiments, the steroid treatment is an ophthalmic steroid treatment. In some embodiments, the ophthalmic steroid treatment is a difluprednate treatment. In some embodiments, the steroid treatment is administered before, during, and/or after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered during administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered after

administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before and during administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before and after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered during, and after administration of the unit dose of rAAV particles. In some embodiments, the steroid treatment is administered before, during, and after administration of the unit dose of rAAV particles.

[0113] In some embodiments, the steroid treatment is an ophthalmic steroid treatment. In some embodiments, the ophthalmic steroid treatment is a daily steroid treatment for up to 4 weeks, up to 6 weeks, or up to 8 weeks after administration of the unit dose of rAAV particles. In some embodiments, 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 embodiments, the ophthalmic steroid treatment is extended at the discretion of the treating physician.

[0114] In some embodiments, the ophthalmic steroid is about 0.005% to about 0.5% difluprednate. In some embodiments, the ophthalmic steroid is any of about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009%, about 0.01%, about 0.02%, about 0.03%, about 0.4%, about 0.05%, about 0.06%, about 0.07%, about 0.08%, about 0.09%, or about 0.1% difluprednate. In some embodiments, the ophthalmic steroid is difluprednate 0.05%. In some embodiments, a dose of difluprednate 0.05% is one drop of ophthalmic solution. In some embodiments, one drop is about 50 μ l (e.g., about 25 μ l to about 50 μ l, about 50 μ l to about 100 μ l). In some embodiments, a dose of difluprednate comprises about 1 μ g to about 5 μ g, or about 2 μ g to about 3 μ g, or about 2.5 μ g difluprednate. In some embodiments, a dose of difluprednate comprises about 2.5 μ g difluprednate.

Vectors for Delivering Transgenes to Target Cells

[0115] In some embodiments, the recombinant adeno-associated virus (rAAV) particles comprise a recombinant viral vector derived from adeno-associated virus (AAV) that has been altered so that it is replication-defective in the subject (e.g., a human or a non-human primate). In some embodiments, the adeno-associated virus (AAV) is a recombinant AAV (rAAV).

[0116] AAV or rAAV are small non-enveloped single-stranded DNA viruses. rAAVs are non-pathogenic human parvoviruses and can be made to be dependent on helper viruses, including adenovirus, herpes simplex virus, vaccinia virus and CMV, for replication.

[0117] Exposure to wild type (wt) AAV is not associated or known to cause any human pathologies and is common in the general population, making AAV or rAAV a suitable delivery system for gene therapy. AAV and rAAV used for gene therapy for delivery of an anti-VEGF agent, e.g., aflibercept, can be of any serotype. In some embodiments, the methods of the disclosure provide for use of any suitable AAV serotype, including AAV1, AAV2, AAV2.5, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, rh10, AAV-DJ, and any hybrid or chimeric AAV thereof. In some

embodiments, the serotype used is based on tropism of the virus, or infectivity of a target cell of interest. In some embodiments, several AAV vectors may be generated to allow selection of the most optimal serotype for use with an anti-VEGF agent transgene (e.g., aflibercept transgene).

[0118] In some embodiments, the methods of the present disclosure provide for the use of pseudotyped AAV. Pseudotyped AAV particles comprise AAV genome inverted terminal repeats (ITRs) of one AAV serotype encapsidated by an AAV capsid of another AAV serotype. Typically, pseudotyped AAV is designated as “AAV#/#”, where the first “#” indicates the AAV ITR serotype and the second “#” indicates capsid serotype. For example, an AAV particle comprising AAV2 ITRs and an AAV1 capsid would be designated “AAV2/1”.

[0119] In some embodiments, the rAAV particles comprise a nucleic acid, e.g., a heterologous nucleic acid. In some embodiments, the nucleic acid encodes a transgene, e.g., an anti-VEGF agent (e.g., aflibercept). In some embodiments, the encoded transgene, e.g., anti-VEGF agent, is under the transcriptional control of a promoter that initiates transcription of the nucleic acid. In some embodiments, the promoter is a “ubiquitous” promoter. In some embodiments, the promoter is a “strong” or constitutively active promoter, e.g., a cytomegalovirus (CMV) promoter, an elongation factor 1 alpha (EF1a) promoter, a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) promoter, or a connexin36 (or “Cx36”) promoter. In some embodiments, the promoter is a tissue-specific promoter that is activated in specific tissues or cells, such as retinal cells, to reduce potential toxicity or undesirable effects to non-targeted cells. In some aspects, several AAV vectors may be generated to allow selection of the most optimal serotype and promoter for use with the anti-VEGF agent transgene (e.g., aflibercept transgene). In some embodiments, the nucleic acid is flanked by AAV inverted terminal repeats (ITRs). In some embodiments, the nucleic acid is flanked by AAV2 ITRs.

[0120] In some embodiments, the AAV vector comprises a polynucleotide cassette for enhanced expression of a transgene (e.g., an anti-VEGF agent such as aflibercept) in a target cell (e.g., a retinal cell). In some embodiments, the polynucleotide cassette comprises in 5' to 3' order: (a) a first enhancer region comprising a CMV sequence (SEQ ID NO: 22); (b) a promoter region, comprising a CMV sequence (SEQ ID NO: 23); (c) a 5'UTR region comprising, in 5' to 3' order, TPL and eMLP sequences (SEQ ID NO: 24 and SEQ ID NO: 25, respectively); (d) a coding sequence encoding a peptide or polypeptide (e.g., an anti-VEGF agent such as aflibercept); (e) a second enhancer region comprising a full EES sequence (SEQ ID NO: 26); and (f) a HGH polyadenylation site (SEQ ID NO: 27). In certain of these embodiments, the polynucleotide cassette comprises one or more sequences selected from SEQ ID NO: 28-32 or a sequence with at least 85% identity thereto. In certain of these embodiments, the 5' arm of the polynucleotide cassette comprises or consists of SEQ ID NO: 33 or a sequence with at least 85% identity thereto. In certain of these embodiments, the 3' arm of the polynucleotide cassette comprises or consists of SEQ ID NO: 34 or a sequence with at least 85% identity thereto. The nucleic acid sequences of SEQ ID NOs: 22-34 are provided below:

ACTTACGGTA AATGGCCCCG CTGGCTGACC GCCCAACGAC CCCC GCCCAT TGACGTCAAT
 AATGACGTAT GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC AATGGGTGGA
 GTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG TATCATATGC CAAGTCCGCC
 CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT TATGCCCAGT ACATGACCTT
 ACGGGACTTT CCTACTTGGC AGTACATCTA CGTATTAGTC ATCGCTATTA CCA (SEQ ID NO:
 22)

TGCTGATGCG GTTTTGGCAG TACACCAATG GCGTGGATA GCGGTTTGAC TCACGGGGAT
 TTCCAAGTCT CCACCCCAT GACGTCAATG GGAGTTTGTT TTGGCACCAA AATCAACGGG
 ACTTTCCAAA ATGTCGTAAT AACCCCGCCC CGTTGACGCA AATGGGCGGT AGGCGTGTAC
 GGTGGGAGGT CTATATAAGC AGAGCTCGTT TAGTGAACCG (SEQ ID NO: 23)

CTCACTCTCT TCCGCATCGC TGTCTGCGAG GGCCAGCTGT TGGGCTCGCG GTTGAGGACA
 AACTCTTCGC GGTCTTTCCA GTACTCTTGG ATCGGAAACC CGTCGGCCTC CGAACGGTAC
 TCCGCCACCG AGGGACCTGA GCGAGTCCGC ATCGACCGGA TCGGAAACC TCTCGAGAAA
 GCGCTCTAAC CAGTCACAGT CGCAAGGTAG GCTGAGCACC GTGGCGGGCG GCAGCGGGTG
 GCGGTGCGGG TTGTTTCTGG CGGAGGTGCT GCTGATGATG TAATTAAAGT AGGCGGTCTT
 GAGACGGCGG ATGGTCGA (SEQ ID NO: 24)

CCAGCTGTTG GGGTGAGTAC TCCCTCTCAA AAGCGGGCAT TACTTCTGCG CTAAGATTGT
 CAGTTTCCAA AAACGAGGAG GATTGATAT TCACCTGGCC CG (SEQ ID NO: 25)

CTGTTCTCAT CACATCATAT CAAGGTTATA TACCATCAAT ATTGCCACAG ATGTTACTTA
 GCCTTTTAAAT ATTTCTCTAA TTTAGTGTAT ATGCAATGAT AGTTCTCTGA TTTCTGAGAT
 TGAGTTTCTC ATGTGTAATG ATTATTTAGA GTTCTCTTT CATCTGTCA AATTTTTGTC
 TAGTTTTATT TTTTACTGAT TTGTAAGACT TCTTTTATA ATCTGCATAT TACAATCTC
 TTTACTGGGG TGTTGCAAAT ATTTTCTGTC ATTCTATGGC CTGACTTTTC TTAATGGTTT
 TTTAATTTTA AAAATAAGTC TTAATATTCA TGCAATCTAA TTAACAATCT TTTCTTTGTG
 GTTAGGACTT TGAGTCATAA GAAATTTTTC TCTACACTGA AGTCATGATG GCATGCTTCT
 ATATTATTTT CTAAAAGATT TAAAGTTTTG CCTTCTCCAT TTAGACTTAT AATTCAGTGG
 AATTTTTTTG TGTGTATGGT ATGACATATG GGTTCCCTTT TATTTTTTAC ATATAAATAT
 ATTTCCCTGT TTTTCTAAAA AAGAAAAAGA TCATCATTTT CCCATTGTAA AATGCCATAT
 TTTTTTCTA GGTCACTTAC ATATATCAAT GGGTCTGTTT CTGAGCTCTA CTCTATTTTA
 TCAGCCTCAC TGTCTATCCC CACACATCTC ATGCTTTGCT CTAATCTTG ATATTTAGTG
 GAACATTCTT TCCCATTTTG TTCTACAAGA ATATTTTGT TATTGTCTTT GGGCTTTCTA
 TATACATTTT GAAATGAGGT TGACAAGTTA (SEQ ID NO: 26)

CTGCCCCGGT GGCATCCCTG TGACCCCTCC CCAGTGCCCT TCCTGGCCCT GGAAGTTGCC
 ACTCCAGTGC CCACCAGCCT TGTCTAATA AAATTAAGTT GCATCATTTT GTCTGACTAG
 GTGTCCTTCT ATAATATTAT GGGGTGGAGG GGGGTGGTAT GGAGCAAGGG GCCCAAGTTG
 GGAAGAAACC TGTAGGCCT GC (SEQ ID NO: 27)

AGGCGGTCTT GAGACGGCGG ATGGTCGAGG TGAGGTGTGG CAGGCTTGAG ATCCAGCTGT
 TGGGGTGA (SEQ ID NO: 28)

CGCTGTTTTG ACCTCCATAG TGGACACCGG GACCGATCCA GCCTCCGCGT CTCAGGGGAG
 ATCTCGTTTA GTGAACCGTC AGATCCTCAC TCTCTCCGC ATCGCTGTCT GCGAGGGCCA
 GCTGTTGGG (SEQ ID NO: 29)

TTGATATTCA CCTGGCCCCG TCTGGCCATA CACTTG (SEQ ID NO: 30)

CCCAGGTCCA AGTTTAAACG CC (SEQ ID NO: 31)

TCTTTGGGCT TTCTATATAC ATTTTGAAAT GAGGTGACA AGTTACCTAG GAAACTGTC
 TTCCTGCCCC GGTGGCA (SEQ ID NO: 32)

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CTCTGGAGAC GACTTACGGT AAATGGCCCCG CCTGGCTGAC CGCCCAACGA CCCCCGCCCCA
TTGACGTCAA TAATGACGTA TGTTCCCATTA GTAACGCCAA TAGGGACTTT CCATTGACGT
CAATGGGTGG AGTATTTACG GTAAACTGCC CACTTGGCAG TACATCAAGT GTATCATATG
CCAAGTCCGC CCCCTATTGA CGTCAATGAC GGTAAATGGC CCGCCTGGCA TTATGCCCAG
TACATGACCT TACGGGACTT TCCTACTTGG CAGTACATCT ACGTATTAGT CATCGCTATT
ACCATGCTGA TGCGGTTTTG GCAGTACACC AATGGGCGTG GATAGCGGTT TGACTCACGG
GGATTTCCAA GTCTCCACCC CATTGACGTC AATGGGAGTT TGTTTTGGCA CCAAAATCAA
CGGGACTTTC CAAAATGTCG TAATAACCCC GCCCGTTGA CGCAAATGGG CGGTAGGCGT
GTACGGTGGG AGGTCTATAT AAGCAGAGCT CGTTTAGTGA ACCGTCAGAT CGCCTGGAGA
GGCCATCCAC GCTGTTTTGA CCTCCATAGT GGACACCGGG ACCGATCCAG CCTCCGCGTC
TCAGGGGAGA TCTCGTTTAG TGAACCGTCA GATCCTCACT CTCTTCCGCA TCGCTGTCTG
CGAGGGCCAG CTGTTGGGCT CGCGGTTGAG GACAACTCT TCGCGGTCTT TCCAGTACTC
TTGGATCCGA AACCCGTCGG CCTCCGAACG GTACTCCGCC ACCGAGGGAC CTGAGCGAGT
CCGCATCGAC CGGATCGGAA AACCTCTCGA GAAAGGCGTC TAACCACTCA CAGTCGCAAG
GTAGGCTGAG CACCGTGGCG GGCGGCAGCG GGTGGCGGTC GGGGTTGTTT CTGGCGGAGG
TGCTGCTGAT GATGTAATTA AAGTAGGCGG TCTTGAGACG GCGGATGGTC GAGGTGAGGT
GTGGCAGGCT TGAGATCCAG CTGTTGGGGT GAGTACTCCC TCTCAAAGC GGGCATTACT
TCTGCGCTAA GATTGTCAGT TTCCAAAAAC GAGGAGGATT TGATATTCAC CTGGCCCGAT
CTGGCCATAC ACTTGAGTGA CAATGACATC CACTTTGCCT TTCTCTCCAC AGGTGTCCAC
TCCCAGGTCC AAGTTTAAAC GCCGCCACCA TG (SEQ ID NO: 33)

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ACTGTTCTCA TCACATCATA TCAAGGTTAT ATACCATCAA TATTGCCACA GATGTTACTT
AGCCTTTTAA TATTTCTCTA ATTTAGTGTA TATGCAATGA TAGTTCTCTG ATTTCTGAGA
TTGAGTTTCT CATGTGTAAT GATTATTTAG AGTTTCTCTT TCATCTGTTC AAATTTTTGT
CTAGTTTTAT TTTTTACTGA TTTGTAAGAC TTCTTTTAT AATCTGCATA TTACAATTCT
CTTTACTGGG GTGTTGCAAA TATTTTCTGT CATCTATGG CCTGACTTTT CTTAATGGTT
TTTTAATTTT AAAAATAAGT CTTAATATTC ATGCAATCTA ATTAACAATC TTTTCTTTGT
GGTTAGGACT TTGAGTCATA AGAAATTTTT CTCTACACTG AAGTCATGAT GGCATGCTTC
TATATTATTT TCTAAAAGAT TTAAAGTTT CCCTTCTCCA TTAGACTTA TAATTCAGT
GAATTTTTTT GTGTGTATGG TATGACATAT GGGTTCCCTT TTATTTTTTA CATATAAATA
TATTTCCCTG TTTTTCTAAA AAAGAAAAAG ATCATCATTT TCCCATTGTA AAATGCCATA
TTTTTTTCAT AGGTCACCTA CATATATCAA TGGGTCTGTT TCTGAGCTCT ACTCTATTTT
ATCAGCCTCA CTGTCTATCC CCACACATCT CATGCTTTGC TCTAAATCTT GATATTTTAGT
GGAACATTCT TTCCCATTTT GTTCTACAAG AATATTTTTG TTATTGTCTT TGGGCTTTCT
ATATACATTT TGAATGAGG TTGACAAGTT ACCTAGGAAA ACTGTCTTCC TGCCCGGGTG
GCATCCCTGT GACCCCTCCG CAGTGCCTCT CCTGGCCCTG GAAGTTGCCA CTCCAGTGCC
CACCAGCCTT GTCCTAATAA AATTAAGTTG CATCATTTTG TCTGACTAGG TCTCCTTCTA
TAATATTATG GGGTGGAGGG GGGTGGTATG GAGCAAGGGG CCCAAGTTGG GAAGAAACCT
GTAGGGCCTG CGAAGACAGT CAG (SEQ ID NO: 34)

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[0121] Additional polynucleotide cassettes for enhanced expression of a transgene (e.g., a transgene encoding an anti-VEGF agent such as aflibercept) in a target cell (such as a retinal cell) are disclosed in WO2018/170473, the contents of which related to polynucleotide cassettes for enhanced expression of a transgene in a target cell are incorporated herein by reference.

[0122] In some embodiments, the rAAV particles comprise a variant capsid protein having increased infectivity of target cells, e.g. retinal cells, are used to increase transduction of retinal cells or to increase targeting of gene delivery to retinal cells in an individual. In some embodiments, the rAAV particle comprises an amino acid modification in a capsid protein GH loop/loop IV of the AAV capsid protein. In some embodiments, the site of modification is a solvent-accessible portion of the GH loop/loop IV of the AAV capsid protein. For a description of the GH loop/loop IV of AAV capsid, see, e.g., van Vliet et al. (2006) *Mol. Ther.* 14:809; Padron et al. (2005) *J. Virol.* 79:5047; and Shen et al. (2007) *Mol. Ther.*

15:1955. Several AAV capsid variants are known, including the 7m8 variant. In some embodiments, a rAAV particle comprises a variant AAV capsid protein that comprises an insertion of from 5 amino acids to 11 amino acids, e.g., 7 amino acid sequence, in the GH loop of a capsid protein relative to a corresponding parental AAV capsid protein, and wherein the variant capsid protein confers increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV particle comprising the corresponding parental or unmodified AAV capsid protein. In some embodiments, any one of the following amino acid sequences can be inserted in the GH loop of a capsid protein: LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8), LAKDTDTTTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), and LASTGKVPNA (SEQ ID NO: 12), LGETTRP (SEQ ID NO: 14), NETITRP (SEQ ID NO: 15), KAGQANN (SEQ ID NO: 16), KDPKTTN (SEQ ID NO: 17), KDTDTTR (SEQ ID NO: 18), RAGGSVG (SEQ ID NO: 19), AVDTTKF (SEQ ID NO: 20), and STGKVPN (SEQ ID NO: 21). In some embodiments, any one of the amino acid sequences set forth in SEQ ID NOs: 1-12 and 14-21 is inserted in the solvent-exposed GH loop of VP1 capsid protein in a rAAV. Additional details regarding amino acid sequences that can be inserted into the GH loop of a capsid protein, e.g., to facilitate transduction of a nucleic acid of interest to a retinal cell following IVT injection, are provided in WO2012145601, US9587282, US10202657, and US10214785, the contents of which related to amino acid sequences that can be inserted into the GH loop of a capsid protein are incorporated herein by reference.

[0123] In some embodiments, the rAAV particles comprise an AAV capsid protein, e.g., an AAV2 capsid protein, that includes any one of the following amino acid sequences: LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8), LAKDTDTTTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), and LASTGKVPNA (SEQ ID NO: 12), LGETTRP (SEQ ID NO: 14), NETITRP (SEQ ID NO: 15), KAGQANN (SEQ ID NO: 16), KDPKTTN (SEQ ID NO: 17), KDTDTTR (SEQ ID NO: 18), RAGGSVG (SEQ ID NO: 19), AVDTTKF (SEQ ID NO: 20), and STGKVPN (SEQ ID NO: 21) inserted at the following positions: between positions 587 and 588 of the AAV2 capsid protein; between amino acids 590 and 591 of the AAV1 capsid protein; between amino acids 575 and 576 of the AAV5 capsid protein; between amino acids 590 and 591 of the AAV6 capsid protein; between amino acids 589 and 590 of the AAV7 capsid protein; between amino acids 590 and 591 of the AAV8 capsid protein; between amino acids 588 and 589 of the AAV9 capsid protein; or between amino acids 589 and 590 of the AAV10 capsid protein. In some embodiments, the rAAV particles comprise AAV2 capsid proteins comprising an 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 embodiments, the rAAV particles comprise AAV2 capsid proteins

comprising 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.

[0124] In some embodiments, rAAV particles comprise the 7m8 variant capsid protein from AAV2 comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the AAV2 VP1. The sequence of the 7m8 variant capsid protein from AAV2 comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted between positions 587 and 588 of the AAV2 VP1 is provided below:

IHSLPIVSDWLEDTLSEGIRQWWKLKPGPPPKPAERHKDDSRGLVLPGYKYLGPFNGLDKGEPV
NEADAAALEHDKAYDRQLDSGDNPYLKYNHADADEFQERLKEDTSFGGNLGRAVFQAKKRVL
LGLVEEPVKTA PGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPQPLGQPP
AAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTTSTRTWALPTYNN
HLYKQISSQSGASNDNHYFGYSTPWGYDFDNRFHCHFSRPDWQLINNNWGFPRKRLNFKLFNI
QVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNN
GSQAVGRSSFYCLEYFPSQMLRTGNNTFSYTFEDVPFHSSY AHSQSLDRLMNPLIDQYLYLSR
TNTPSGTTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLN
GRDSL VNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQY
GSVSTNLQRGNLALGETTRPARQAATADVNTQGVLPGMVWQDRDVYLGPIWAKIPHTDGHFH
PSPLMGGFGLKHPPQILIKNTVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENS KRWN
EIQYTSNYNKS VNVDFTVDTNGVYSEPRPIGTRYLTRNL (SEQ ID NO: 37)

[0125] In some embodiments, the rAAV particles comprise a capsid protein VP1 comprising the 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 embodiments, the rAAV particles comprise a capsid protein VP2 comprising the 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 embodiments, the rAAV particles comprise a capsid protein VP3 comprising the 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 embodiments, the rAAV particles comprise capsid proteins VP1, VP2, and VP3, wherein each of VP1, VP2, and VP3 comprise the 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.

[0126] In some embodiments, the rAAV particles comprise a capsid protein VP1 comprising 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 embodiments, the rAAV particles comprise a capsid protein VP2 comprising 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 embodiments, the rAAV particles comprise a capsid protein VP3 comprising 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 embodiments, the rAAV particles comprise capsid proteins VP1, VP2, and VP3, wherein each of VP1, VP2, and VP3 comprise 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.

[0127] In some embodiments, a recombinant virus and/or plasmid used to generate a rAAV virus comprises other transcriptional or regulatory elements, such as a poly A (polyadenylation) sequence, untranslated regions (UTRs), 3' UTRs, or termination sequences. In some embodiments, more than one gene is expressed from the vector or plasmid using internal ribosome entry site (IRES) or similar element that allows co-expression of two or more proteins or create multigene, or polycistronic mRNA.

[0128] In some embodiments, the rAAV and/or plasmid used to generate the rAAV comprises one or more of the following nucleic acid elements: a first ITR sequence; a promoter sequence; an intron sequence; a first UTR sequence; a heterologous nucleic acid encoding an anti-VEGF agent (e.g., aflibercept); a second UTR sequence; a polyA sequence; and a second ITR sequence. In some embodiments, linker sequence(s) are inserted between two or more of the nucleic acid elements. In some embodiments, the heterologous nucleic acid encodes a therapeutic polypeptide encodes aflibercept (or a functional fragment or functional variant thereof).

[0129] In some embodiments, the vector is a targeted vector, especially a targeted rAAV (e.g., AAV2.7m8) that shows higher infectivity of a specific cell, such as a retinal cell (e.g., a photoreceptor, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelium cell). Viral vectors for use in the disclosure can include those that exhibit low toxicity and/or low immunogenicity in an individual and expresses therapeutically effective quantities of the anti-VEGF agent (e.g., aflibercept) in an individual, e.g., a human. Any suitable method known in the art can be used in the biochemical purification of recombinant viruses (e.g., rAAV), e.g., for the preparation of pharmaceutical compositions described elsewhere herein. Recombinant AAV viruses can be harvested directly from cells, or from the culture media comprising cells. Virus can be purified using various biochemical means, such as gel filtration, filtration, chromatography, affinity purification, gradient ultracentrifugation, or size exclusion methods. In some embodiments, the virus is lyophilized.

[0130] In some embodiments, the rAAV particles comprise a 7m8 variant capsid protein, e.g., rAAV2.7m8, and a nucleic acid sequence that encodes an anti-VEGF agent (e.g., aflibercept, or a functional fragment or functional variant thereof). In some embodiments, the rAAV particles (e.g., the 7m8 variant) have an increase in retinal cell infectivity of any of at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein. In some embodiments, the increase in infectivity of retinal cells is an increase of any of between

5% to 100%, between 5% to 95%, between 5% to 90%, between 5% to 85%, between 5% to 80%, between 5% to 75%, between 5% to 70%, between 5% to 65%, between 5% to 60%, between 5% to 55%, between 5% to 50%, between 5% to 45%, between 5% to 40%, between 5% to 35%, between 5% to 30%, between 5% to 25%, between 5% to 20%, between 5% to 15%, between 5% to 10% as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0131] In some embodiments, the increase in retinal cell infectivity of a rAAV variant, e.g., rAAV2.7m8, is any of at least 1-fold, at least 1.1-fold, at least 1.2-fold, at least 1.3-fold, at least 1.4-fold, at least 1.5-fold, at least 1.6-fold, at least 1.7-fold, at least 1.8-fold, at least 1.9-fold, or at least 2-fold compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein. In some embodiments, the increase in infectivity is any of at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold as compared to an AAV particle comprising the corresponding parental AAV capsid protein. In some embodiments, the increase in infectivity is any of at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 35-fold, at least 40-fold, at least 45-fold, at least 50-fold, at least 55-fold, at least 60-fold, at least 65-fold, at least 70-fold, at least 75-fold, at least 80-fold, at least 85-fold, at least 90-fold, or at least 100-fold compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0132] In some embodiments, the increase in retinal cell infectivity of a rAAV variant, e.g., rAAV2.7m8, is between 10-fold to 100-fold, between 10-fold to 95-fold, between 10-fold to 90-fold, between 10-fold to 85-fold, between 10-fold to 80-fold, between 10-fold to 75-fold, between 10-fold to 70-fold, between 10-fold to 65-fold, between 10-fold to 60-fold, between 10-fold to 55-fold, between 10-fold to 50-fold, between 10-fold to 45-fold, between 10-fold to 40-fold, between 10-fold to 35-fold, between 10-fold to 30-fold, between 10-fold to 25-fold, between 10-fold to 20-fold, or between 10-fold to 15-fold as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0133] In some embodiments, the increase in retinal cell infectivity is between 2-fold to 20-fold, between 2-fold to 19-fold, between 2-fold to 18-fold, between 2-fold to 17-fold, between 2-fold to 16-fold, between 2-fold to 15-fold, between 2-fold to 14-fold, between 2-fold to 13-fold, between 2-fold to 12-fold, between 2-fold to 11-fold, between 2-fold to 10-fold, between 2-fold to 9-fold, between 2-fold to 8-fold, between 2-fold to 7-fold, between 2-fold to 6-fold, between 2-fold to 5-fold, between 2-fold to 4-fold, or between 2-fold to 3-fold as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0134] In some embodiments, an amino acid modification of a capsid protein described herein can confer an increase in an ability to cross an internal limiting membrane (ILM) in an eye of an individual, e.g., a human, as compared to the ability of an AAV particle comprising the corresponding parental or unmodified AAV capsid protein to cross the ILM in the eye of the subject. In some embodiments, the increase in the ability to cross the ILM of a rAAV variant, e.g., rAAV2.7m8, is an increase of any of at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at

least 80%, at least 90%, or at least 100% as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein. In some embodiments, the increase in the ability to cross the ILM is an increase of between 5% to 100%, between 5% to 95%, between 5% to 90%, between 5% to 85%, between 5% to 80%, between 5% to 75%, between 5% to 70%, between 5% to 65%, between 5% to 60%, between 5% to 55%, between 5% to 50%, between 5% to 45%, between 5% to 40%, between 5% to 35%, between 5% to 30%, between 5% to 25%, between 5% to 20%, between 5% to 15%, or between 5% to 10% as compared to the parental or unmodified AAV capsid protein.

[0135] In some embodiments, the increase in the ability to cross the ILM of a rAAV variant, e.g., rAAV2.7m8, is any of at least 1-fold, at least 1.1-fold, at least 1.2-fold, at least 1.3-fold, at least 1.4-fold, at least 1.5-fold, at least 1.6-fold, at least 1.7-fold, at least 1.8-fold, at least 1.9-fold, or at least 2-fold compared to an AAV particle comprising the corresponding parental AAV capsid protein. In some embodiments, the increase in the ability to cross the ILM is any of at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold as compared to an AAV particle comprising the corresponding parental AAV capsid protein. In some embodiments, the increase in the ability to cross the ILM is any of at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 35-fold, at least 40-fold, at least 45-fold, at least 50-fold, at least 55-fold, at least 60-fold, at least 65-fold, at least 70-fold, at least 75-fold, at least 80-fold, at least 85-fold, at least 90-fold, or at least 100-fold compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0136] In some embodiments, the increase in the ability to cross the ILM of a rAAV variant, e.g., rAAV2.7m8, is between 10-fold to 100-fold, between 10-fold to 95-fold, between 10-fold to 90-fold, between 10-fold to 85-fold, between 10-fold to 80-fold, between 10-fold to 75-fold, between 10-fold to 70-fold, between 10-fold to 65-fold, between 10-fold to 60-fold, between 10-fold to 55-fold, between 10-fold to 50-fold, between 10-fold to 45-fold, between 10-fold to 40-fold, between 10-fold to 35-fold, between 10-fold to 30-fold, between 10-fold to 25-fold, between 10-fold to 20-fold, or between 10-fold to 15-fold as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0137] In some embodiments, the increase in the ability to cross the ILM of a rAAV variant, e.g., rAAV2.7m8, is between 2-fold to 20-fold, between 2-fold to 19-fold, between 2-fold to 18-fold, between 2-fold to 17-fold, between 2-fold to 16-fold, between 2-fold to 15-fold, between 2-fold to 14-fold, between 2-fold to 13-fold, between 2-fold to 12-fold, between 2-fold to 11-fold, between 2-fold to 10-fold, between 2-fold to 9-fold, between 2-fold to 8-fold, between 2-fold to 7-fold, between 2-fold to 6-fold, between 2-fold to 5-fold, between 2-fold to 4-fold, or between 2-fold to 3-fold as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0138] In some embodiments, rAAV.7m8 comprising nucleic acid encoding aflibercept is used for gene therapy. In some embodiments, AAV2 or rAAV2 is used to deliver a nucleic acid sequence encoding an anti-VEGF agent (e.g., aflibercept) into an eye or retinal cells of a subject via intravitreal or

subretinal injection. In some embodiments, AAV2 or rAAV2 is used to deliver a nucleic acid sequence encoding an anti-VEGF agent (e.g., aflibercept) into an eye or retinal cells of a subject via intravitreal injection. In some embodiments, rAAV2.7m8 is used to deliver the nucleic acid sequence of the anti-VEGF agent (e.g., aflibercept) into the retinal cells of a subject. In some embodiments, the heterologous nucleic acid (e.g., a nucleic acid that encodes an anti-VEGF agent such as aflibercept) integrates into the target cell genome (e.g., retinal cell genome), resulting in long-term expression of, e.g., the anti-VEGF agent (such as aflibercept), in the target cell. In some embodiments, the viral vector delivers a plasmid or other extrachromosomal genetic element that comprises the heterologous nucleic acid (e.g., a nucleic acid that encodes an anti-VEGF agent such as aflibercept) to the target cell (e.g., retinal cell).

[0139] In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising an amino acid sequence with any of at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise 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). In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the rAAV particles comprise a nucleic acid encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 35. In some embodiments, the rAAV particles comprise a nucleic acid encoding aflibercept and flanked by AAV2 inverted terminal repeats (ITRs). The sequence of SEQ ID NO: 35 is provided below:

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SDTGRPFVEMYSEIPEIIHMTGRELVI PCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFII SNATY
KEIGLLTCEATVNGHLYKTNLYLTHRQTNTIIDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPS
SKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPP
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSL
SLSPG (SEQ ID NO: 35)
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[0140] In some embodiments, the rAAV particles comprise a nucleic acid with any of at least about 75%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.9%, or at least about 100% sequence homology to the nucleic acid sequence of SEQ ID NO: 36, and wherein the nucleic acid is flanked by AAV2 inverted terminal repeats (ITRs). The sequence of SEQ ID NO: 36 is

provided in FIG. 5. In some embodiments, the rAAV particles comprise a nucleic acid with any of at least about 75%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.9%, or at least about 100% sequence homology to the nucleic acid sequence of aflibercept (e.g., SEQ ID NO: 36), and wherein the nucleic acid is flanked by AAV2 inverted terminal repeats (ITRs). In some embodiments, the nucleic acid sequence of aflibercept is derived from its amino acid sequence. In some embodiments, the nucleic acid sequence of aflibercept is codon optimized to improve its expression in a subject.

[0141] In some embodiments, the nucleic acid sequence of aflibercept is codon-optimized for expression in a primate or a human subject. Construction of a synthetic gene corresponding to the aflibercept amino acid sequence has been described in literature, e.g., Kanda A, Noda K, Saito W, Ishida S. Aflibercept Traps Galectin-1, an Angiogenic Factor Associated with Diabetic Retinopathy. *Scientific Reports* 5:17946 (2015) (describing “VEGF-Trap_{R1R2} (corresponding to aflibercept) cDNA was generated as a synthetic gene by IDT (Coralville, IA)”). Given the available amino acid sequence of aflibercept, any method known in the art can be used to generate the cDNA of aflibercept for use in a gene therapy or a rAAV described herein.

[0142] Codon optimization can be achieved with any method known in the art. Codon optimization refers to a process of modifying a nucleic acid sequence for enhanced expression of a gene in target or host cells of interest, e.g., human retinal cells, by replacing at least one codon (e.g., about or more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100 or more codons) of a native sequence with codons that are used more frequently or are most frequently used in the host cell while maintaining the native amino acid sequence. Codon usage tables are readily available, including for examples, GenScript Codon Usage Frequency Table Tool at [www\(dot\)genscript\(dot\)com/tools/codon-frequency-table](http://www(dot)genscript(dot)com/tools/codon-frequency-table); Codon Usage Database at [www\(dot\)kazusa\(dot\)or\(dot\)jp/codon/](http://www(dot)kazusa(dot)or(dot)jp/codon/); and Nakamura, Y., et al. “Codon usage tabulated from the international DNA sequence databases: status for the year 2000” *Nucl. Acids Res.* 28:292 (2000).

[0143] Homology refers to the percent conservation of residues of an alignment between two sequences, including, but not limited to functional fragments, sequences comprising insertions, deletions, substitutions, pseudofragments, pseudogenes, splice variants or artificially optimized sequences.

[0144] In some embodiments, the rAAV particles comprise a nucleic acid encoding aflibercept. In some embodiments, the polypeptide is aflibercept.

[0145] As used herein, “aflibercept” refers to a polypeptide or protein sequence, or a functional fragment or variant or mutant thereof, with any of at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more, or 100% homology to the aflibercept amino acid sequence identified above (SEQ ID NO: 35). Homology refers to the percent conservation of residues of an alignment between two sequences, including, but not limited to functional

fragments, sequences comprising insertions, deletions, substitutions, pseudofragments, pseudogenes, splice variants or artificially optimized sequences.

[0146] In some embodiments, the amino acid sequence of aflibercept is any of at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% homologous to the aflibercept amino acid sequence of SEQ ID NO: 35. In some embodiments, the nucleic acid sequence encoding aflibercept disclosed herein is compared to the corresponding cDNA sequence of the aflibercept amino acid sequence identified above, and shows any of at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% sequence homology between the nucleic acid sequences of aflibercept (e.g., SEQ ID NO: 36). In some embodiments, aflibercept is any of at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% spatially homologous to aflibercept (e.g., in terms of its secondary, tertiary, and quaternary structure or conformation). In some embodiments, aflibercept is any of at most 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% spatially homologous to the aflibercept used in the standard of care (e.g., secondary, tertiary, and quaternary structure or conformation).

[0147] In some embodiments, the aflibercept gene product, or aflibercept transgene, as included in a gene therapy based on a rAAV, comprises a capsid variant as disclosed herein (e.g., the 7m8 variant), encodes a protein, fusion protein, or polypeptide that has any of at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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 at least 100% homology to the above amino acid sequence of SEQ ID NO: 35, or between the corresponding cDNA sequences of aflibercept (e.g., cDNA of aflibercept sequence used in a gene therapy compared to SEQ ID NO: 36). In some embodiments, the methods compositions disclosed herein comprise a functional fragment of aflibercept, or a variant or mutant thereof. In some embodiments, the nucleic acid sequence of aflibercept is modified or codon-optimized to enhance its activity, expression, stability, and/or solubility *in vivo*.

[0148] Aflibercept is a 115 kDa fusion protein, which can be glycosylated. Aflibercept comprises an IgG backbone fused to extracellular VEGF receptor sequences of the human VEGFR-1 and VEGFR-2, and functions like a soluble decoy receptor by binding VEGF-A with a greater affinity than its natural or endogenous receptors. See, for example, Stewart MW. Aflibercept (VEGF Trap-eye): the newest anti-VEGF drug. *Br. J. Ophthalmol.* 2012 Sep;96(9):1157-8. Aflibercept's high affinity for VEGF interferes or disrupts subsequent binding and activation of native or endogenous VEGF receptors. Reduced VEGF activity can lead to decreased angiogenesis and vascular permeability. Inhibition of placental growth factor PlGF and VEGF-B by aflibercept may also contribute to the treatment of ocular diseases or disorders characterized by abnormal (e.g., excessive) angiogenesis and/or neovascularization. PlGF has been associated with angiogenesis and certain ocular diseases or disorders, such as wet AMD, may be associated with elevated levels of PlGF. VEGF-B overexpression can be associated with breakdown of

the blood-retinal barrier and retinal angiogenesis. Thus, inhibition of VEGF-A, VEGF-B, and PlGF may all contribute to the efficacy of aflibercept.

Methods for Preparation of Vectors for Delivering Transgenes to Target Cells

[0149] In some embodiments, the rAAV particles are manufactured using any method known in the art. In some embodiments, the rAAV particles are manufactured using a baculovirus expression vector system in Sf9 cells. Sf9 cells are an insect cell culture cell line commonly used for recombinant protein production using baculovirus. In some embodiments, the rAAV particles are manufactured using two baculoviruses in Sf9 cells. In some embodiments, the rAAV particles are manufactured using two baculoviruses in Sf9 cells, wherein a first baculovirus encodes the genes for AAV2 Rep and AAV2.7m8 Cap proteins and a second baculovirus encodes an anti-VEGF agent. In some embodiments, the rAAV particles are manufactured using two baculoviruses in Sf9 cells, wherein a first baculovirus encodes the genes for AAV2 Rep and AAV2.7m8 Cap proteins and a second baculovirus encodes an aflibercept (e.g., human aflibercept) cDNA expression cassette. In some embodiments, the rAAV particles are manufactured using two baculoviruses in Sf9 cells, wherein a first baculovirus encodes the genes for AAV2 Rep and AAV2.7m8 Cap proteins and a second baculovirus comprises 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). In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 35. In some embodiments, the polypeptide is aflibercept.

Doses

[0150] In some embodiments, the unit dose of rAAV particles is administered to one eye of the individual. In some embodiments, the one eye of the individual is the right eye or the left eye. In some embodiments, the one eye of the individual is the right eye. In some embodiments, the one eye of the individual is the left eye. In some embodiments, the methods provided herein further comprise administering a unit dose of rAAV particles to the contralateral eye of the individual. In some embodiments, the one eye of the individual is the right eye and the contralateral eye is the left eye. In some embodiments, the one eye of the individual is the left eye and the contralateral eye is the right eye.

[0151] In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye is at least about 2 weeks (e.g., at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about 7 months, at least about 8 months, at least about 9 months, at least about 10 months, at least about 11 months, at least about 12 months, at least about 1 year, at least about 2 years, at least about 3 years, at least about 4 years, at least about 5 years, or more) after administering the unit dose of rAAV particles to the one eye. In some embodiments, 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 and the unit dose of rAAV particles administered to the contralateral eye of the individual is higher (e.g., more than any of about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300% or more) than the unit dose of rAAV particles administered to the one eye of the individual.

[0152] In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye of the individual is up to about 1 week, up to about 2 weeks, up to about 3 weeks, or up to about 4 weeks after administering the unit dose of rAAV particles to the one eye. In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye of the individual is up to about 2 weeks (e.g., about 0 days, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days) after administering the unit dose of rAAV particles to the one eye. In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye of the individual is up to about 2 weeks (e.g., about 0 days, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days) after administering the unit dose of rAAV particles to the one eye and the unit dose of rAAV particles administered to the contralateral eye of the individual is about the same (e.g., less than 1% higher or lower, less than 5% higher or lower, less than 10% higher or lower, or less than 20% higher or lower) or lower (e.g., about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% lower) than the unit dose of rAAV particles administered to the one eye of the individual. In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye of the individual is up to about 2 weeks after administering the unit dose of rAAV particles to the one eye and the unit dose of rAAV particles administered to the contralateral eye of the individual is about the same (e.g., less than 1% higher or lower, less than 5% higher or lower, less than 10% higher or lower, or less than 20% higher or lower) as the unit dose of rAAV particles administered to the one eye of the individual. In some embodiments, the administering the unit dose of rAAV particles to the contralateral eye of the individual is up to about 2 weeks after administering the unit dose of rAAV particles to the one eye and the unit dose of rAAV particles administered to the contralateral eye of the individual is lower (e.g., about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% lower) than the unit dose of rAAV particles administered to the one eye of the individual.

[0153] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles is expressed as the number of vector genomes (vg). In some embodiments, the unit dose is about 6×10^{11} vector genomes (vg) or less of the rAAV particles. In some embodiments, the unit dose is about 1×10^{10} to about 2×10^{10} , between about 2×10^{10} to about 3×10^{10} , between about 3×10^{10} to about 4×10^{10} , between about 4×10^{10} to about 5×10^{10} , between about 5×10^{10} to about 6×10^{10} , between about 6×10^{10} to about 7×10^{10} , between about 7×10^{10} to about 8×10^{10} , between about 8×10^{10} to about 9×10^{10} , between about 9×10^{10} to about 10×10^{10} , between about 1×10^{11} to about 2×10^{11} , between about 2×10^{11} to about 3×10^{11} , between about

3×10^{11} to about 4×10^{11} , between about 4×10^{11} to about 5×10^{11} , or between about 5×10^{11} to about 6×10^{11} vg of the rAAV particles, including any value within these ranges, of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vector genomes (vg) to about 2×10^{11} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg to about 2×10^{11} vg, about 7×10^{10} vg to about 2×10^{11} vg, about 8×10^{10} vg to about 2×10^{11} vg, about 9×10^{10} vg to about 2×10^{11} vg, about 10×10^{10} vg to about 2×10^{11} vg, or about 1×10^{11} vg to about 2×10^{11} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg to about 2×10^{11} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg to about 7×10^{10} vg, about 7×10^{10} vg to about 8×10^{10} vg, about 8×10^{10} vg to about 9×10^{10} vg, about 9×10^{10} vg to about 10×10^{10} vg, about 10×10^{10} vg to about 1×10^{11} vg, or about 1×10^{11} vg to about 2×10^{11} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg, about 7×10^{10} vg, about 8×10^{10} vg, about 9×10^{10} vg, about 10×10^{10} vg, about 1×10^{11} vg, or about 2×10^{11} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg or about 2×10^{11} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg, about 2×10^{11} vg, or about 6×10^{11} vg. In some embodiments, the unit dose is about 6×10^{10} vg. In some embodiments, the unit dose is about 2×10^{11} vg. In some embodiments, the unit dose is about 6×10^{11} vg.

[0154] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose is expressed as the number of vector genomes (vg) per eye (vg/eye). In some embodiments, the unit dose is about 6×10^{11} vg/eye or less of the rAAV particles. In some embodiments, the unit dose is about 1×10^{10} to about 2×10^{10} , between about 2×10^{10} to about 3×10^{10} , between about 3×10^{10} to about 4×10^{10} , between about 4×10^{10} to about 5×10^{10} , between about 5×10^{10} to about 6×10^{10} , between about 6×10^{10} to about 7×10^{10} , between about 7×10^{10} to about 8×10^{10} , between about 8×10^{10} to about 9×10^{10} , between about 9×10^{10} to about 10×10^{10} , between about 1×10^{11} to about 2×10^{11} , between about 2×10^{11} to about 3×10^{11} , between about 3×10^{11} to about 4×10^{11} , between about 4×10^{11} to about 5×10^{11} , or between about 5×10^{11} to about 6×10^{11} vg/eye of the rAAV particles, including any value within these ranges, of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye to about 2×10^{11} vg/eye, about 7×10^{10} vg/eye to about 2×10^{11} vg/eye, about 8×10^{10} vg/eye to about 2×10^{11} vg/eye, about 9×10^{10} vg/eye to about 2×10^{11} vg/eye, about 10×10^{10} vg/eye to about 2×10^{11} vg/eye, or about 1×10^{11} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye to about 7×10^{10} vg/eye, about 7×10^{10} vg/eye to about 8×10^{10} vg/eye, about 8×10^{10} vg/eye to about 9×10^{10} vg/eye, about 9×10^{10} vg/eye to about 10×10^{10} vg/eye, about 10×10^{10} vg/eye to about 1×10^{11} vg/eye, or about 1×10^{11} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye, about 7×10^{10} vg/eye, about 8×10^{10} vg/eye, about 9×10^{10} vg/eye, about 10×10^{10} vg/eye, about 1×10^{11} vg/eye, or about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose is about 6×10^{10} vg/eye or about

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

[0155] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, E is a shorthand for base 10 for exponentiation, and xE_y refers to x multiplied by base 10 to the y power/exponent. In some embodiments, the unit dose is expressed as the number of vector genomes (vg). In some embodiments, the unit dose is about $6E^{11}$ vector genomes (vg) or less of the rAAV particles. In some embodiments, the unit dose is about $1E^{10}$ to about $2E^{10}$, between about $2E^{10}$ to about $3E^{10}$, between about $3E^{10}$ to about $4E^{10}$, between about $4E^{10}$ to about $5E^{10}$, between about $5E^{10}$ to about $6E^{10}$, between about $6E^{10}$ to about $7E^{10}$, between about $7E^{10}$ to about $8E^{10}$, between about $8E^{10}$ to about $9E^{10}$, between about $9E^{10}$ to about $10E^{10}$, between about $1E^{11}$ to about $2E^{11}$, between about $2E^{11}$ to about $3E^{11}$, between about $3E^{11}$ to about $4E^{11}$, between about $4E^{11}$ to about $5E^{11}$, or between about $5E^{11}$ to about $6E^{11}$ vg of the rAAV particles, including any value within these ranges, of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vector genomes (vg) to about $2E^{11}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg to about $2E^{11}$ vg, about $7E^{10}$ vg to about $2E^{11}$ vg, about $8E^{10}$ vg to about $2E^{11}$ vg, about $9E^{10}$ vg to about $2E^{11}$ vg, about $10E^{10}$ vg to about $2E^{11}$ vg, or about $1E^{11}$ vg to about $2E^{11}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg to about $2E^{11}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg to about $7E^{10}$ vg, about $7E^{10}$ vg to about $8E^{10}$ vg, about $8E^{10}$ vg to about $9E^{10}$ vg, about $9E^{10}$ vg to about $10E^{10}$ vg, about $10E^{10}$ vg to about $1E^{11}$ vg, or about $1E^{11}$ vg to about $2E^{11}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg, about $7E^{10}$ vg, about $8E^{10}$ vg, about $9E^{10}$ vg, about $10E^{10}$ vg, about $1E^{11}$ vg, or about $2E^{11}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg or about $2E^{11}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg, about $2E^{11}$ vg, or about 6×10^{11} vg. In some embodiments, the unit dose is about $6E^{10}$ vg. In some embodiments, the unit dose is about $2E^{11}$ vg. In some embodiments, the unit dose is about 6×10^{11} vg.

[0156] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose is expressed as the number of vector genomes (vg) per eye (vg/eye). In some embodiments, the unit dose is about $6E^{11}$ vg/eye or less of the rAAV particles. In some embodiments, the unit dose is about $1E^{10}$ to about $2E^{10}$, between about $2E^{10}$ to about $3E^{10}$, between about $3E^{10}$ to about $4E^{10}$, between about $4E^{10}$ to about $5E^{10}$, between about $5E^{10}$ to about $6E^{10}$, between about $6E^{10}$ to about $7E^{10}$, between about $7E^{10}$ to about $8E^{10}$, between about $8E^{10}$ to about $9E^{10}$, between about $9E^{10}$ to about $10E^{10}$, between about $1E^{11}$ to about $2E^{11}$, between about $2E^{11}$ to about $3E^{11}$, between about $3E^{11}$ to about $4E^{11}$, between about $4E^{11}$ to about $5E^{11}$, or between about $5E^{11}$ to about $6E^{11}$ vg/eye of the rAAV particles, including any value within these ranges, of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye to about $2E^{11}$ vg/eye of the rAAV particles. In

some embodiments, the unit dose is about $6E^{10}$ vg/eye to about $2E^{11}$ vg/eye, about $7E^{10}$ vg/eye to about $2E^{11}$ vg/eye, about $8E^{10}$ vg/eye to about $2E^{11}$ vg/eye, about $9E^{10}$ vg/eye to about $2E^{11}$ vg/eye, about $10E^{10}$ vg/eye to about $2E^{11}$ vg/eye, or about $1E^{11}$ vg/eye to about $2E^{11}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye to about $2E^{11}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye to about $7E^{10}$ vg/eye, about $7E^{10}$ vg/eye to about $8E^{10}$ vg/eye, about $8E^{10}$ vg/eye to about $9E^{10}$ vg/eye, about $9E^{10}$ vg/eye to about $10E^{10}$ vg/eye, about $10E^{10}$ vg/eye to about $1E^{11}$ vg/eye, or about $1E^{11}$ vg/eye to about $2E^{11}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye, about $7E^{10}$ vg/eye, about $8E^{10}$ vg/eye, about $9E^{10}$ vg/eye, about $10E^{10}$ vg/eye, about $1E^{11}$ vg/eye, or about $2E^{11}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye or about $2E^{11}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye of the rAAV particles. In some embodiments, the unit dose is about $6E^{10}$ vg/eye, about $2E^{11}$ vg/eye, or about 6×10^{11} vg/eye. In some embodiments, the unit dose is about $6E^{10}$ vg/eye. In some embodiments, the unit dose is about $2E^{11}$ vg/eye. In some embodiments, the unit dose is about 6×10^{11} vg/eye.

[0157] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) in the vitreous fluid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) at about any one of 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10 μ g/ml, or more, including any range in between these values, in the vitreous fluid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of aflibercept in the vitreous fluid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of aflibercept at about any one of 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10 μ g/ml, or more, including any range in between these values, in the vitreous fluid.

[0158] In some embodiments, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye of the individual is a unit dose sufficient to cause expression of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) in the aqueous fluid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) of at least about 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0 μ g/ml, or more, including any range in between these values, in the aqueous fluid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of aflibercept in the aqueous fluid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of aflibercept of at least about 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0 μ g/ml, or more, including any range in between these values, in the aqueous fluid.

[0159] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles is a unit dose

sufficient to cause expression of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) in the retina. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) of at least about 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10 $\mu\text{g/g}$, or more, including any range in between these values, in the retina. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of aflibercept in the retina. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of aflibercept of at least about 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10 $\mu\text{g/g}$, or more, including any range in between these values, in the retina.

[0160] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) in the choroid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) at about any one of 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10 $\mu\text{g/g}$, or more, including any range in between these values, in the choroid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to cause expression of aflibercept in the choroid. In some embodiments, the unit dose of rAAV particles is a unit dose sufficient to achieve a concentration of aflibercept at about any one of 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10 $\mu\text{g/g}$, or more, including any range in between these values, in the choroid.

[0161] In some embodiments, the unit dose of rAAV particles is administered to one eye and/or to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose.

[0162] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause maintenance or a decrease of retinal thickness compared to the retinal thickness prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause a decrease of retinal thickness compared to the retinal thickness prior to administration of the unit dose of rAAV particles. In some embodiments, retinal thickness is central subfield thickness (CST) or central retinal thickness (CRT). In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause a decrease of retinal thickness of more than any of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or about 100% compared to the retinal thickness prior to administration of the unit dose of rAAV particles. In some embodiments, the retinal thickness (e.g., CST or CRT) is determined by OCT or SD-OCT.

[0163] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause maintenance or a decrease in macular volume compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of

rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause a decrease in macular volume compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause a decrease in macular volume of more than any of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, or about 65% compared to the macular volume prior to administration of the unit dose of rAAV particles. In some embodiments, the macular volume is determined by OCT or SD-OCT.

[0164] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause maintenance or an improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause an improvement of visual acuity compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause an improvement of visual acuity of more than any of about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300%, or more compared to the visual acuity prior to administration of the unit dose of rAAV particles. In some embodiments, visual acuity is best corrected visual acuity (BCVA). In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause an improvement of BCVA compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, BCVA is expressed as an ETDRS score, which corresponds to the number of letters correctly read (Vitale *et al.*, (2016) JAMA Ophthalmol 134(9):1041:1047). In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause an improvement of BCVA of at least 15 ETDRS letters (Vitale *et al.*, (2016) JAMA Ophthalmol 134(9):1041:1047) (e.g., at least about 15, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, or about 70 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if the unit dose is sufficient to cause maintenance of BCVA, wherein the individual loses fewer than 15 ETDRS letters (Vitale *et al.*, (2016) JAMA Ophthalmol 134(9):1041:1047) (e.g., 15 or less, 14 or less, 13 or less, 12 or less, 11 or less, 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, 2 or less, 1, or 0 letters) compared to the BCVA prior to administration of the unit dose of rAAV particles.

[0165] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined to have maintenance of vision. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined have an improvement of vision. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after

administration of the unit dose of rAAV particles, the CST or CRT assessed by SD-OCT is decreased compared to prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the CST or CRT assessed by SD-OCT is maintained compared to prior to administration of the unit dose of rAAV particles.

[0166] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the macular volume is decreased compared to prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the macular volume is maintained compared to prior to administration of the unit dose of rAAV particles.

[0167] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the retinal thickness (e.g., central retinal thickness (CRT) or central subfield thickness (CST)) and macular volume are decreased compared to prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the retinal thickness (e.g., central retinal thickness (CRT) or central subfield thickness (CST)) and macular volume are maintained compared to prior to administration of the unit dose of rAAV particles.

[0168] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual requires less than one rescue therapy treatment (e.g., aflibercept injection) about any of every 4 weeks, every 5 weeks, every 6 weeks, every 7 weeks, every 8 weeks, every 9 weeks, every 10 weeks, or more after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual does not require any rescue therapy treatment (e.g., aflibercept injection) for at least about any of 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 15 weeks, at least 20 weeks, at least 30 weeks, at least 40 weeks, at least 50 weeks, at least 60 weeks, at least 70 weeks, at least 80 weeks, at least 90 weeks, at least 100 weeks, at least 110 weeks, or more.

[0169] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined to have a reduction in retinal fluid compared to the level of retinal fluid prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined to have maintenance in retinal fluid compared to the level of retinal fluid prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined to have a reduction in IRF and/or SRF in the

one eye and/or the contralateral eye compared to the levels of IRF and/or SRF prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0170] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined to have a resolution of pigment epithelial detachment (PED) compared to PED prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0171] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, CNV lesions shrink compared to CNV lesions present prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, CNV lesions shrink by more than any of about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% compared to CNV lesions present prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, CNV lesions do not grow compared to CNV lesions present prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, CNV lesions do not grow by more than about any of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, or 20% compared to CNV lesions present prior to administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye.

[0172] In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined to have an improvement in anatomical features of the one eye and/or the contralateral eye compared to the anatomical features prior to administration of the unit dose of rAAV particles. In some embodiments, the unit dose of rAAV particles is a therapeutically effective dose if, after administration of the unit dose of rAAV particles, the individual is determined to have a stabilization and/or maintenance of anatomical features of the one eye and/or the contralateral eye compared to the anatomical features prior to administration of the unit dose of rAAV particles.

[0173] In some embodiments, the unit dose of rAAV particles is therapeutically effective if administration of the dose to the one eye and/or the contralateral eye of the individual reduces, stops, or prevents at least one symptom of the ocular neovascular disease or disorder. In the cases of ocular neovascular diseases or disorders characterized by abnormal (e.g., excessive) angiogenesis, such symptoms include, but are not limited to, e.g., visual distortions (such as impaired color vision, blurred vision, deterioration of central vision) and vision loss. In some embodiments, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye of the individual is a therapeutically effective dose if administration of the unit dose to the one eye and/or to the contralateral eye of the individual results in the maintenance, partial resolution, or complete resolution of one or more clinical

features of the ocular neovascular disease. For example, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye of the individual is therapeutically effective if administration of the dose to the one eye and/or to the contralateral eye of the individual results in complete resolution, partial resolution or maintenance of the ocular neovascular disease as measured by any method known in the art. In some embodiments, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye of the individual is therapeutically effective if administration of the dose to the one eye and/or to the contralateral eye of the individual results in complete resolution, partial resolution or maintenance of the ocular neovascular disease as assessed by best corrected visual acuity (BCVA) (e.g., based on an ETDRS score; Vitale *et al.*, (2016) JAMA Ophthalmol 134(9):1041:1047), central retinal thickness is determined by SD-OCT, the number of rescue therapy treatments (e.g., aflibercept injections) required by the individual after administration of the unit dose of rAAV particles in the one eye and/or the contralateral eye, the presence of intraretinal fluid (IRF) and/or subretinal fluid (SRF), the resolution of pigment epithelial detachment (PED), choroidal neovascularization (CNV) lesion growth, anatomical features based on any methods known in the art (e.g., SD-OCT, OCT, fluorescein angiography, digital color fundus photography, etc.). In some embodiments, the unit dose of rAAV particles administered to the one eye and/or to the contralateral eye of the individual is therapeutically effective if administration of the dose to the one eye and/or to the contralateral eye of the individual results in complete resolution, partial resolution or maintenance of the ocular neovascular disease as assessed by ophthalmologic examination, intraocular pressure (e.g., using a Goldmann applanation tonometer or Tono-pen), indirect ophthalmoscopy, examination of the one eye and/or the contralateral eye and adnexa, eyelid and/or pupil responsiveness, belpharoptosis, abnormal pupil shape, unequal pupils, abnormal reaction to light, afferent pupillary defects, slit-lamp examination (including of the eyelids, conjunctiva, cornea, lens, iris, and anterior chamber), posterior segment abnormalities of the vitreous, optic nerve, peripheral retina, and retinal vasculature, SD-OCT, fluorescein angiography, digital color fundus photography (including images of the retina, optic disc, and/or macula), aqueous humor sampling, vitreous humor sampling, OCT-angiography (OCT-A), refraction and visual acuity (BCVA).

[0174] In some embodiments, the unit dose of rAAV particles administered to the one eye of the individual is the same as the unit dose of rAAV particles administered to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles administered to the one eye of the individual is different from the unit dose of rAAV particles administered to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles administered to the one eye of the individual is higher, e.g., more than any of about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300% or more, than the unit dose of rAAV particles administered to the contralateral eye of the individual. In some embodiments, the unit dose of rAAV particles administered to the contralateral eye of the individual is higher, e.g., more than any of about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%,

about 100%, about 125%, about 150%, about 175%, about 200%, about 225%, about 250%, about 275%, about 300% or more, than the unit dose of rAAV particles administered to the one eye of the individual. In some embodiments, the unit dose of rAAV particles is expressed as the number of vector genomes (vg) per eye (vg/eye). In some embodiments, the unit dose of rAAV particles is about 6×10^{11} vg/eye or less of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 1×10^{10} to about 2×10^{10} , between about 2×10^{10} to about 3×10^{10} , between about 3×10^{10} to about 4×10^{10} , between about 4×10^{10} to about 5×10^{10} , between about 5×10^{10} to about 6×10^{10} , between about 6×10^{10} to about 7×10^{10} , between about 7×10^{10} to about 8×10^{10} , between about 8×10^{10} to about 9×10^{10} , between about 9×10^{10} to about 10×10^{10} , between about 1×10^{11} to about 2×10^{11} , between about 2×10^{11} to about 3×10^{11} , between about 3×10^{11} to about 4×10^{11} , between about 4×10^{11} to about 5×10^{11} , or between about 5×10^{11} to about 6×10^{11} vg/eye of the rAAV particles, including any value within these ranges, of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye to about 2×10^{11} vg/eye, about 7×10^{10} vg/eye to about 2×10^{11} vg/eye, about 8×10^{10} vg/eye to about 2×10^{11} vg/eye, about 9×10^{10} vg/eye to about 2×10^{11} vg/eye, about 10×10^{10} vg/eye to about 2×10^{11} vg/eye, or about 1×10^{11} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye to about 7×10^{10} vg/eye, about 7×10^{10} vg/eye to about 8×10^{10} vg/eye, about 8×10^{10} vg/eye to about 9×10^{10} vg/eye, about 9×10^{10} vg/eye to about 10×10^{10} vg/eye, about 10×10^{10} vg/eye to about 1×10^{11} vg/eye, or about 1×10^{11} vg/eye to about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye, about 7×10^{10} vg/eye, about 8×10^{10} vg/eye, about 9×10^{10} vg/eye, about 10×10^{10} vg/eye, about 1×10^{11} vg/eye, or about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye or about 2×10^{11} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye of the rAAV particles. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye, about 2×10^{11} vg/eye, or about 6×10^{11} vg/eye. In some embodiments, the unit dose of rAAV particles is about 6×10^{10} vg/eye. In some embodiments, the unit dose of rAAV particles is about 2×10^{11} vg/eye. In some embodiments, the unit dose of rAAV particles is about 6×10^{11} vg/eye.

[0175] In some embodiments, the unit dose of rAAV particles administered to the one eye of the individual and the unit dose of rAAV particles administered to the contralateral eye of the individual are administered at the same time. In some embodiments, the unit dose of rAAV particles administered to the one eye of the individual and the unit dose of rAAV particles administered to the contralateral eye of the individual are administered at different times. In some embodiments, the unit dose administered to the contralateral eye is administered any of at least about 1 hour, at least about 2 hours, at least about 4 hours, at least about 8 hours, at least about 12 hours, at least about 24 hours, at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about

7 days, at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, or more after administering of the unit dose to the one eye. In some embodiments, the unit dose administered to the contralateral eye is administered at least about 2 weeks after administering of the unit dose to the one eye.

[0176] In some embodiments, a single unit dose of rAAV particles is administered to the one eye and/or the contralateral eye of the individual. In some embodiments, the single unit dose of rAAV particles administered to the one eye and/or to the contralateral eye is a therapeutically effective dose. In some embodiments, more than one dose of rAAV particles (e.g., more than any of about 2, 3, 4, 5, or more unit doses) are administered to the one eye and/or the contralateral eye of the individual. In some embodiments, the more than one doses of rAAV particles administered to the one eye and/or to the contralateral are therapeutically effective doses.

Pharmaceutical Formulations

[0177] In some embodiments, the unit dose of rAAV particles is in a pharmaceutical formulation. In some embodiments, the pharmaceutical formulation comprises the rAAV particles, one or more osmotic or ionic strength agents, one or more buffering agents, one or more surfactants, and one or more solvents. In some embodiments, the osmotic or ionic strength agent is sodium chloride. In some embodiments, the one or more buffering agents are sodium phosphate monobasic and/or sodium phosphate dibasic. In some embodiments, the surfactant is Poloxamer 188. In some embodiments, the solvent is water. In some embodiments, the pharmaceutical formulation comprises the rAAV particles, sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic, and a surfactant.

[0178] In some embodiments, the pharmaceutical formulation comprises about 1×10^{10} vg/mL to about 1×10^{13} vg/mL of rAAV particles, about 150 mM to about 200 mM sodium chloride, about 1 mM to about 10 mM monobasic sodium phosphate, about 1 mM to about 10 mM dibasic sodium phosphate, and about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL to about 6×10^{12} vg/mL of rAAV particles, about 150 mM to about 200 mM sodium chloride, about 1 mM to about 10 mM monobasic sodium phosphate, about 1 mM to about 10 mM dibasic sodium phosphate, and about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL of rAAV particles, about 150 mM to about 200 mM sodium chloride, about 1 mM to about 10 mM monobasic sodium phosphate, about 1 mM to about 10 mM dibasic sodium phosphate, and about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5. In some embodiments, the pharmaceutical formulation comprises about 6×10^{12} vg/mL of rAAV particles, about 150 mM to about 200 mM sodium chloride, about 1 mM to about 10 mM monobasic sodium phosphate, about 1 mM to about 10 mM dibasic sodium phosphate, and about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5.

[0179] In some embodiments, the rAAV particles in the pharmaceutical formulation are present at a concentration of about 1×10^{10} vg/ml to about 1×10^{13} vg/ml. In some embodiments, the rAAV particles in the pharmaceutical formulation are present at a concentration of about 1×10^{09} vg/ml to about 6×10^{14} vg/ml. In certain embodiments, the rAAV particles in the pharmaceutical formulation are present at a concentration of about 1×10^{09} vg/ml to about 2×10^{09} vg/ml, about 2×10^{09} vg/ml to about 3×10^{09} , about 3×10^{09} vg/ml to about 4×10^{09} , about 4×10^{09} vg/ml to about 5×10^{09} , about 5×10^{09} vg/ml to about 6×10^{09} , about 6×10^{09} vg/ml to about 7×10^{09} , about 7×10^{09} vg/ml to about 8×10^{09} , about 8×10^{09} vg/ml to about 9×10^{09} , about 9×10^{09} vg/ml to about 10×10^{09} , about 10×10^{09} vg/ml to about 1×10^{10} , about 1×10^{10} vg/ml to about 2×10^{10} , about 2×10^{10} vg/ml to about 3×10^{10} , about 3×10^{10} vg/ml to about 4×10^{10} , about 4×10^{10} vg/ml to about 5×10^{10} , about 5×10^{10} vg/ml to about 6×10^{10} , about 6×10^{10} vg/ml to about 7×10^{10} , about 7×10^{10} vg/ml to about 8×10^{10} , about 8×10^{10} vg/ml to about 9×10^{10} , about 9×10^{10} vg/ml to about 10×10^{10} , about 10×10^{10} vg/ml to about 1×10^{11} , about 1×10^{11} vg/ml to about 2×10^{11} , about 2×10^{11} vg/ml to about 3×10^{11} , about 3×10^{11} vg/ml to about 4×10^{11} , about 4×10^{11} vg/ml to about 5×10^{11} , about 5×10^{11} vg/ml to about 6×10^{11} , about 6×10^{11} vg/ml to about 7×10^{11} , about 7×10^{11} vg/ml to about 8×10^{11} , about 8×10^{11} vg/ml to about 9×10^{11} , about 9×10^{11} vg/ml to about 10×10^{11} , about 1×10^{12} vg/ml to about 2×10^{12} , about 2×10^{12} vg/ml to about 3×10^{12} , about 3×10^{12} vg/ml to about 4×10^{12} , about 4×10^{12} vg/ml to about 5×10^{12} , about 5×10^{12} vg/ml to about 6×10^{12} , about 6×10^{12} vg/ml to about 7×10^{12} , about 7×10^{12} vg/ml to about 8×10^{12} , about 8×10^{12} vg/ml to about 9×10^{12} , about 9×10^{12} vg/ml to about 10×10^{12} , about 1×10^{13} vg/ml to about 2×10^{13} , about 2×10^{13} vg/ml to about 3×10^{13} , about 3×10^{13} vg/ml to about 4×10^{13} , about 4×10^{13} vg/ml to about 5×10^{13} , about 5×10^{13} vg/ml to about 6×10^{13} , about 6×10^{13} vg/ml to about 7×10^{13} , about 7×10^{13} vg/ml to about 8×10^{13} , about 8×10^{13} vg/ml to about 9×10^{13} , about 9×10^{13} vg/ml to about 10×10^{13} , about 1×10^{14} vg/ml to about 2×10^{14} , about 2×10^{14} vg/ml to about 3×10^{14} , about 3×10^{14} vg/ml to about 4×10^{14} , about 4×10^{14} vg/ml to about 5×10^{14} , or about 5×10^{14} vg/ml to about 6×10^{14} vg/mL. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL to about 6×10^{12} vg/mL of rAAV particles. In some embodiments, the pharmaceutical formulation comprises about 6×10^{12} vg/mL of rAAV particles. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL of rAAV particles. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL to about 6×10^{12} vg/mL of rAAV particles. In some embodiments, the pharmaceutical formulation comprises about 6×10^{12} vg/mL of rAAV particles. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL of rAAV particles.

[0180] In some embodiments, the sodium chloride in the pharmaceutical formulation is present at a concentration of about 150 mM to about 200 mM. In certain embodiments, the sodium chloride in the pharmaceutical formulation is present at a concentration of about 150 mM, about 160 mM, about 170 mM, about 180 mM, about 190 mM, or about 200 mM. In certain embodiments, the sodium chloride in the pharmaceutical formulation is present at a concentration of about 180 mM.

[0181] In some embodiments, the sodium phosphate monobasic is present in the pharmaceutical formulation at a concentration of about 1 mM to about 10 mM. In some embodiments, the sodium

phosphate monobasic is present in the pharmaceutical formulation at a concentration of any of about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, or about 10 mM. In certain embodiments, the sodium phosphate monobasic is present in the pharmaceutical formulation at a concentration of about 5 mM.

[0182] In some embodiments, the sodium phosphate dibasic is present in the pharmaceutical formulation at a concentration of about 1 mM to about 10 mM. In some embodiments, the sodium phosphate dibasic is present in the pharmaceutical formulation at a concentration of any of about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, or about 10 mM. In certain embodiments, the sodium phosphate dibasic is present in the pharmaceutical formulation at a concentration of about 5 mM.

[0183] In some embodiments, the Poloxamer 188 is present in the pharmaceutical formulation at a concentration of about 0.0005% (w/v) to about 0.005% (w/v). In some embodiments, the Poloxamer 188 is present in the pharmaceutical formulation at a concentration of any of about 0.0005% (w/v), about 0.0006% (w/v), about 0.0007% (w/v), about 0.0008% (w/v), about 0.0009% (w/v), about 0.001% (w/v), about 0.002% (w/v), about 0.003% (w/v), about 0.004% (w/v), or about 0.005% (w/v). In certain embodiments, the Poloxamer 188 is present in the pharmaceutical formulation at a concentration of about 0.001% (w/v).

[0184] In some embodiments, the pharmaceutical formulation has a pH of about 7.0 to about 7.5. In some embodiments, the pharmaceutical formulation has a pH of about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, or about 7.5. In certain embodiments, the pharmaceutical formulation has a pH of about 7.3. In some embodiments, hydrochloric acid and sodium hydroxide are used to adjust the pH of the pharmaceutical formulation.

[0185] In some embodiments, the pharmaceutical formulation comprises about 6×10^{12} vg/mL of rAAV particles, about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3. In some embodiments, the pharmaceutical formulation comprises about 6×10^{11} vg/mL of rAAV particles, about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3.

[0186] In some embodiments, the pharmaceutical formulations are suitable for administration to the one eye and/or the contralateral eye of the individual, e.g., a human patient, via intravitreal (IVT) injection to achieve a desired therapeutic or prophylactic effect. In some embodiments, the pharmaceutical formulation is supplied as a reconstituted homogenous solution. In some embodiments, the solution is a suspension. In some embodiments, the pharmaceutical formulation is supplied as a frozen suspension, and is thawed prior to administration to the one eye and/or the contralateral eye of the individual. In some embodiments, the solution is isotonic.

[0187] In other embodiments, the pharmaceutical composition comprising e.g., an AAV2.7m8 vector that comprises a nucleic acid sequence encoding the anti-VEGF agent (e.g., a functional fragment or variant thereof), is supplied in a lyophilized form, and is reconstituted prior to administration to the one eye and/or the contralateral eye of the individual. In some embodiments, the methods provided herein further comprise the steps of reconstituting, dissolving, or solubilizing a lyophilized pharmaceutical composition comprising rAAV (e.g., AAV2.7m8) and encoding the anti-VEGF agent (e.g., aflibercept or a functional fragment or variant thereof) in a buffer prior to administration to the subject. In some embodiments, such lyophilized pharmaceutical composition comprises one or more of the following: a cryoprotectant, a surfactant, a salt, a stabilizer, or any combination thereof.

[0188] In some embodiments, the pharmaceutical formulation is a homogenous solution. In some embodiments, the homogenous solution is supplied in a pre-filled syringe. In some embodiments, the pharmaceutical formulation is supplied as a suspension. In some embodiments, a suspension is a solution. In some embodiments, the suspension is refrigerated. In some embodiments, the suspension is frozen. In some embodiments, methods provided herein further comprise the step of warming the refrigerated suspension to room temperature and/or agitating the suspension to ensure that the active ingredient(s) are dissolved and/or evenly distributed in solution prior to administering to the one eye and/or the contralateral eye of the individual (e.g., via IVT injection). In some embodiments, method provided herein further comprise the step of thawing the frozen suspension and warming to room temperature and/or agitating the suspension to ensure that the active ingredient(s) are dissolved and/or evenly distributed in solution prior to administering to the one eye and/or the contralateral eye of the individual (e.g., via IVT injection). In some embodiments, the suspension is diluted prior to administration to the subject (e.g., via IVT injection). In some embodiments, the suspension is supplied as a pre-filled syringe.

[0189] In some embodiments, the pharmaceutical formulation is provided as a frozen suspension. In some embodiments, the suspension comprises a pharmaceutically acceptable excipient, e.g., surfactant, glycerol, non-ionic surfactant, buffer, glycol, salt, and any combination thereof.

[0190] In some embodiments, the suspension is a solution. In some embodiments, the suspension comprises micelles.

[0191] In some embodiments, for storage stability and convenience of handling, a pharmaceutical formulation, comprising rAAV (e.g., AAV2.7m8) and a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept or a functional fragment or variant thereof), is formulated as a lyophilized, freeze dried, or vacuum dried powder that is reconstituted with saline, buffer, or water prior to administration to the one eye and/or the contralateral eye of the individual. Alternately, the pharmaceutical formulation is formulated as an aqueous solution, such as a suspension or a homogeneous solution. A pharmaceutical formulation can contain rAAV particles comprising a nucleic acid sequence that encodes aflibercept. Various excipients, such as phosphate, PBS, or Tris buffer, glycol, glycerol, saline, surfactant (e.g., pluronic or polysorbate), or any combination thereof, can be used to stabilize a pharmaceutical formulation. Additionally, cryoprotectants, such as alcohols can be used as a stabilizer

under freezing or drying conditions. In some embodiments, the gene therapy is provided as a suspension, a refrigerated suspension, or a frozen suspension.

[0192] In some embodiments, a suspension of the pharmaceutical formulation as disclosed herein has a volume of any of about 20 μL , 30 μL , 40 μL , 50 μL , 60 μL , 70 μL , 80 μL , 90 μL , 100 μL , 200 μL , 300 μL , 400 μL , 500 μL , 600 μL , 700 μL , 800 μL , 900 μL , or 1000 μL . In some embodiments, a suspension of the pharmaceutical formulation as disclosed herein has a volume of about 250 μL . In some embodiments, the suspension of the pharmaceutical formulation as disclosed herein has a volume of between 0.1 to 0.5 mL, between 0.1 to 0.2 mL, between 0.3 to 0.5 mL, between 0.5-1.0 mL, between 0.5-0.7 mL, between 0.6 to 0.8 mL, between 0.8 to 1 mL, between 0.9 to 1.1 mL, between 1.0 to 1.2 mL, or between 1.0 to 1.5 mL. In other embodiments, the volume is no more than 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, 0.6 mL, 0.7 mL, 0.8 mL, 0.9 mL, 1.0 mL, 1.1 mL, 1.2 mL, 1.3 mL, 1.4 mL, or 1.5 mL. In some embodiments, the suspension of the pharmaceutical formulation as disclosed herein has a volume of about 0.25 mL.

[0193] In some embodiments, a suspension of the pharmaceutical formulation as disclosed herein is provided as a sterile-filtered, frozen suspension in a sterile, ready-to-use vial (e.g., a 0.5 mL vial; e.g., a Crystal Zenith® vial) with a ready-to-use stopper (e.g., a stopper made of chlorobutyl), and sealed (e.g., with a sterile aluminum tear-off seal). In some embodiments, a suspension of the pharmaceutical formulation as disclosed herein is provided as a sterile-filtered, frozen suspension in a sterile, ready-to-use vial (e.g., a 0.5 mL vial; e.g., a Crystal Zenith® vial) with a ready-to-use stopper (e.g., a stopper made of chlorobutyl), and sealed (e.g., with a sterile aluminum tear-off seal), wherein the vial contains a volume of between 0.1 to 0.5 mL, between 0.1 to 0.2 mL, between 0.2 to 0.3 mL, between 0.3 to 0.4 mL, or between 0.4 mL to 0.5 mL of the suspension of the pharmaceutical formulation. In some embodiments, a suspension of the pharmaceutical formulation as disclosed herein is provided as a sterile-filtered, frozen suspension in a sterile, ready-to-use vial (e.g., a 0.5 mL vial; e.g., a Crystal Zenith® vial) with a ready-to-use stopper (e.g., a stopper made of chlorobutyl), and sealed (e.g., with a sterile aluminum tear-off seal), wherein the vial contains a volume of about 0.25 mL of the suspension of the pharmaceutical formulation.

[0194] In some embodiments, pharmaceutical formulations disclosed herein are designed, engineered, or adapted for administration to a primate (e.g., non-human primate and human subjects) via intravitreal or subretinal injection. In some embodiments, a pharmaceutical formulation comprising rAAV particles comprising a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept) is formulated for intravitreal injection into an eye of an individual. In some embodiments, the pharmaceutical composition is formulated to or reconstituted to a concentration that allows intravitreal injection of a volume not more than about or not more than any of 25 μL , 30 μL , 35 μL , 40 μL , 45 μL , 50 μL , 55 μL , 60 μL , 65 μL , 70 μL , 75 μL , 80 μL , 85 μL , 90 μL , 95 μL , 100 μL , 110 μL , 120 μL , 130 μL , 140 μL , 150 μL , 160 μL , 170 μL , 180 μL , 190 μL , 200 μL , 210 μL , 220 μL , 230 μL , 240 μL , or 250 μL . In some embodiments, a unit dose of the pharmaceutical formulation comprises a volume not more than about or not more than any of 25 μL , 30 μL , 35 μL , 40 μL , 45 μL , 50 μL , 55 μL , 60 μL , 65 μL , 70 μL , 75 μL , 80 μL , 85 μL , 90 μL , 95

μL, 100 μL, 110 μL, 120 μL, 130 μL, 140 μL, 150 μL, 160 μL, 170 μL, 180 μL, 190 μL, 200 μL, 210 μL, 220 μL, 230 μL, 240 μL, or 250 μL. In some embodiments, methods disclosed herein comprise intravitreal injection of a volume of any of about 25 μL, 30 μL, 35 μL, 40 μL, 45 μL, 50 μL, 55 μL, 60 μL, 65 μL, 70 μL, 75 μL, 80 μL, 85 μL, 90 μL, 95 μL, 100 μL, 110 μL, 120 μL, 130 μL, 140 μL, 150 μL, 160 μL, 170 μL, 180 μL, 190 μL, 200 μL, 210 μL, 220 μL, 230 μL, 240 μL, or 250 μL of a solution or suspension of a pharmaceutical formulation comprising a rAAV (e.g., AAV2.7m8) and a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept). In some embodiments, methods disclosed herein comprise intravitreal injection of a volume of about 30 μL or about 100 μL of a solution or suspension of a pharmaceutical formulation comprising a rAAV (e.g., AAV2.7m8) and a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept). In some embodiments, methods disclosed herein comprise intravitreal injection of a volume of about 30 μL of a solution or suspension of a pharmaceutical formulation comprising a rAAV (e.g., AAV2.7m8) and a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept). In some embodiments, methods disclosed herein comprise intravitreal injection of a volume of about 100 μL of a solution or suspension of a pharmaceutical formulation comprising a rAAV (e.g., AAV2.7m8) and a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept).

[0195] In some embodiments, an AAV2.7m8 particle comprising a nucleic acid sequence of the anti-VEGF agent (e.g., aflibercept) transgene described herein is a component of a gene therapy pharmaceutical formulation. In some embodiments, a rAAV particle of any serotype comprising the 7m8 variant capsid protein as described herein is used to make a frozen suspension or a freeze-dried or lyophilized formulation composition. In some embodiments, the gene therapy is formulated as a refrigerated or frozen suspension. In some embodiments, the rAAV particle is rAAV2. In some embodiments, the lyophilized or suspension of the pharmaceutical formulation comprises rAAV2 comprising the 7m8 variant capsid protein and a DNA sequence that encodes the anti-VEGF agent (e.g., aflibercept). In some embodiments, the suspension is refrigerated or frozen.

[0196] In some embodiments, the administration of the unit dose of rAAV particles to the one eye and/or to the contralateral eye of the individual is by intravitreal (IVT) injection. For IVT injection, the rAAV particles can be delivered in the form of a suspension of a pharmaceutical formulation (e.g., as described herein). Initially, topical anesthetic is applied to the surface of the eye followed by an ophthalmic antiseptic solution. The eye is held open, with or without instrumentation, and the rAAV particles are injected through the sclera with a short, narrow needle, e.g., a 30-gauge needle, into the vitreous cavity of the one eye and/or the contralateral eye of the individual under direct observation. Typically, a volume of between about 25 μL to about 250 μL (e.g., any of about 25 μL, about 30 μL, about 40 μL, about 50 μL, about 60 μL, about 70 μL, about 80 μL, about 90 μL, about 100 μL, about 110 μL, about 120 μL, about 130 μL, about 140 μL, about 150 μL, about 160 μL, about 170 μL, about 180 μL, about 190 μL, about 200 μL, about 210 μL, about 220 μL, about 230 μL, about 240 μL, or about 250 μL) of an rAAV particle suspension may be delivered to the eye by IVT injection. In some embodiments, the

unit dose of rAAV particles comprises a volume of about 100 μL . In some embodiments, the unit dose of rAAV particles comprises a volume of about 30 μL . In some embodiments, the IVT injection is performed in combination with removal of vitreous fluid. In some embodiments, a vitrectomy may be performed, and the entire volume of vitreous gel is replaced by an infusion of the rAAV particle suspension (e.g., about 4 mL of the rAAV particle suspension). A vitrectomy is performed using a cannula of appropriate bore size (e.g., 20 gauge to 27 gauge), wherein the volume of vitreous gel that is removed is replaced by infusion of fluid, e.g., saline, an isotonic solution, a rAAV particle suspension, from the infusion cannula. IVT administration is generally well tolerated. At the conclusion of the procedure, there is sometimes mild redness at the injection site. There is occasional tenderness, but most patients do not report any pain. No eye patch or eye shield is necessary after this procedure, and activities are not restricted. Sometimes, an antibiotic eye drop is prescribed for several days to help prevent infection.

[0197] In some embodiments, the pharmaceutical formulation is a unit dose (e.g., a therapeutically effective dose) to be administered to the one eye and/or the contralateral eye of an individual (e.g., a human or non-human primate) via IVT injection for the treatment of an ocular disease or disorder characterized by abnormal (e.g., excessive) angiogenesis or neovascularization. In some embodiments, the pharmaceutical formulation comprises a unit dose (e.g., a therapeutically effective dose) as described in further detail elsewhere herein. In some embodiments, the volume of the unit dose (e.g., a therapeutically effective dose) of a viral vector (e.g., an rAAV vector disclosed herein) administered to the subject is no more than any one of about 25 μL , 30 μL , 35 μL , 40 μL , 45 μL , 50 μL , 55 μL , 60 μL , 65 μL , 70 μL , 75 μL , 80 μL , 85 μL , 90 μL , 95 μL , 100 μL , 110 μL , 120 μL , 130 μL , 140 μL , 150 μL , 160 μL , 170 μL , 180 μL , 190 μL , 200 μL , 210 μL , 220 μL , 230 μL , 240 μL , or 250 μL , including any range in between these values. Minimizing the volume of the unit dose to be administered to the subject may obviate or mitigate changes in ocular pressure and other adverse effects associated with IVT injection (e.g., elevated intraocular pressure, inflammation, irritation, or pain).

[0198] Pharmaceutical formulations suitable for ocular use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions, suspension, or dispersion. For intravitreal administration, suitable carriers include physiological saline, bacteriostatic water, phosphate buffered saline (PBS), and/or an isotonic agent, e.g., glycerol. In certain embodiments, the pharmaceutical formulation is sterile and fluid to the extent that easy syringability or injectability exists. In certain embodiments, the pharmaceutical formulation is stable under the conditions of manufacture and storage and is preserved against the contaminating action of microorganisms such as bacteria and fungi. In some embodiments, the pharmaceutical composition can include an isotonic agent, such as a salt or glycerol. In some embodiments, a surfactant or a stabilizer is added to the pharmaceutical composition to prevent aggregation.

[0199] In some embodiments, the pharmaceutical formulation contains an excipient or a carrier. A carrier is a solvent or dispersion medium containing, for example, water, saline, ethanol, a polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and any combination

thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants such as polysorbates (e.g., Tween™, polysorbate 20, polysorbate 80), sodium dodecyl sulfate (sodium lauryl sulfate), lauryl dimethyl amine oxide, cetyltrimethylammonium bromide (CTAB), polyethoxylated alcohols, polyoxyethylene sorbitan, octoxynol (Triton X100™), N,N-dimethyldodecylamine-N-oxide, hexadecyltrimethylammonium bromide (HTAB), polyoxyl 10 lauryl ether, Brij 721™, bile salts (sodium deoxycholate, sodium cholate), pluronic acids (F-68, F-127), polyoxyl castor oil (Cremophor™) nonylphenol ethoxylate (Tergitol™), cyclodextrins and, ethylbenzethonium chloride (Hyamine™). Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, cresol, thimerosal, and the like. In many embodiments, isotonic agents are included, for example, sugars, polyalcohols such as mannitol, sorbitol, and/or sodium chloride in the pharmaceutical formulation. Prolonged absorption of the internal compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin. In some embodiments, the pharmaceutical carrier includes sodium phosphate, sodium chloride, polysorbate, and sucrose. In some embodiments, a pharmaceutical formulation comprises a surfactant, e.g., non-ionic surfactant such as polysorbate, poloxamer, or pluronic. In some embodiments, the addition of a non-ionic surfactant reduces aggregation in the pharmaceutical composition.

[0200] Also provided herein are kits comprising at least one pharmaceutical formulation described herein. In some embodiments, the kit comprises a frozen suspension of a pharmaceutical formulation (e.g., one unit dose in a vial). In some embodiments, the kit comprises a lyophilized or freeze-dried pharmaceutical formulation (e.g., one unit dose in a vial) disclosed herein and a solution for dissolving, diluting, and/or reconstituting the lyophilized pharmaceutical composition. In some embodiments, the solution for reconstituting or dilution is supplied as a pre-filled syringe. In some embodiments, a kit comprises a freeze-dried or lyophilized pharmaceutical composition comprising rAAV (e.g., AAV2.7m8) and a solution for reconstituting the pharmaceutical composition to a desired concentration or volume. In some embodiments, the kit includes a buffer that helps to prevent aggregation upon reconstituting the pharmaceutical composition disclosed herein. In some embodiments, the pharmaceutical composition is provided in a pre-filled syringe. In some embodiments, a kit comprises a dual-chamber syringe or container wherein one of the chambers contains a buffer for dissolving or diluting the pharmaceutical composition. In some embodiments, the kit comprises a syringe for injection. In some embodiments, the reconstituted solution is filtered before administration. In some embodiments, the kit comprises a filter or a filter syringe for filtering the reconstituted pharmaceutical composition before administration to a patient. In some embodiments, the kit comprises a suspension of the pharmaceutical formulation comprising the rAAV particles as disclosed herein provided as a sterile-filtered, frozen suspension in a sterile, ready-to-use vial (e.g., a 0.5 mL vial; e.g., a Crystal Zenith® vial) with a ready-to-use stopper (e.g., a stopper made of chlorobutyl), and sealed (e.g., with a sterile aluminum tear-off seal). In some

embodiments, the kit comprises a suspension of the pharmaceutical formulation comprising the rAAV particles as disclosed herein provided as a sterile-filtered, frozen suspension in a sterile, ready-to-use vial (e.g., a 0.5 mL vial; e.g., a Crystal Zenith® vial) with a ready-to-use stopper (e.g., a stopper made of chlorobutyl), and sealed (e.g., with a sterile aluminum tear-off seal), wherein the vial contains a volume of between 0.1 to 0.5 mL, between 0.1 to 0.2 mL, between 0.2 to 0.3 mL, between 0.3 to 0.4 mL, or between 0.4 mL to 0.5 mL of the suspension of the pharmaceutical formulation. In some embodiments, the kit comprises a suspension of the pharmaceutical formulation comprising the rAAV particles as disclosed herein provided as a sterile-filtered, frozen suspension in a sterile, ready-to-use vial (e.g., a 0.5 mL vial; e.g., a Crystal Zenith® vial) with a ready-to-use stopper (e.g., a stopper made of chlorobutyl), and sealed (e.g., with a sterile aluminum tear-off seal), wherein the vial contains a volume of about 0.25 mL of the suspension of the pharmaceutical formulation. In some embodiments, the kit further comprises instructions for use; e.g., instructions for treating an ocular neovascular disease with the rAAV particles disclosed herein.

Ocular Neovascular Diseases

[0201] In one aspect, the present disclosure provides methods for treating an ocular neovascular disease in an individual. In another aspect, the present disclosure provides methods for reducing retinal fluid in the eye of an individual with an ocular neovascular disease.

[0202] In some embodiments, the ocular neovascular disease is age-related macular degeneration (AMD), wet-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. In some embodiments, the ocular neovascular disease is active choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD). In some embodiments, the ocular neovascular disease is recurrent and/or persistent wAMD. In some embodiments, the ocular neovascular disease is active subfoveal CNV secondary to AMD. In some embodiments, the active subfoveal CNV secondary to AMD occupies $\geq 50\%$ of the total lesion size. In some embodiments, the active subfoveal CNV secondary to AMD occupies $\geq 50\%$ of the total lesion size with evidence of leakage on fluorescein angiogram (FA), fluid on spectral domain optical coherence tomography (SD-OCT), and/or subretinal hemorrhage on color fundus photography. In some embodiments, the active subfoveal CNV secondary to AMD occupies $\geq 50\%$ of the total lesion size with evidence of leakage on fluorescein angiogram (FA), fluid on spectral domain optical coherence tomography (SD-OCT), and/or subretinal hemorrhage on color fundus photography, and the entire dimension of the lesion does not exceed 12 macular photocoagulation study disc areas. In some embodiments, the one eye and/or the contralateral eye of the individual exhibited best corrected visual acuity (BCVA) based on an ETDRS letters assessment of 78-25 (e.g., less than any of about 78, about 75, about 70, about 65, about 60, about 55, about 50, about 45, about 40, about 35, about 30, or about 25) prior to administration of the unit dose of rAAV particles of the present disclosure. In some embodiments, the one eye and/or the contralateral

eye of the individual exhibited best corrected visual acuity (BCVA) based on an ETDRS letters assessment of more than any of about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, or about 100 prior to administration of the unit dose of rAAV particles of the present disclosure.

[0203] In some embodiments, the individual had polypoidal choroidal vasculopathy (PCV) in the one eye and/or the contralateral eye prior to administration of the unit dose of rAAV particles.

[0204] In some embodiments, ETDRS letters assessment is done at about 0.5 meters, about 1 meter, about 2 meters, about 3 meters, or about 4 meters. In some embodiments, ETDRS letters assessment is done at about 4 meters.

[0205] In some embodiments, the individual received at least one prior treatment (e.g., at least one, at least two, at least three, at least four, at least 5 or more treatments) with an anti-VEGF agent (e.g., bevacizumab, brolocizumab, ranibizumab, faricimab, abicipar pegol, and/or aflibercept) in about the last 12 weeks (e.g., about 4 months) prior to administration of the unit dose of rAAV particles. In some embodiments, the individual received 2 or 3 prior treatments with an anti-VEGF agent (e.g., bevacizumab, brolocizumab, ranibizumab, faricimab, abicipar pegol, and/or aflibercept) in the one eye and/or in the contralateral eye during about the last 12 weeks (e.g., about 4 months) prior to administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye. In some embodiments, the individual received at least about 1, at least about 5, at least about 10, at least about 20, at least about, at least about 40, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, at least about 100, at least about 110, at least about 120, or more prior treatments with an anti-VEGF agent (e.g., bevacizumab, brolocizumab, ranibizumab, faricimab, abicipar pegol, and/or aflibercept) in the one eye and/or the contralateral eye. In some embodiments, the individual had a calculated anti-VEGF agent (e.g., bevacizumab, brolocizumab, ranibizumab, and/or aflibercept) injection interval in the one eye and/or the contralateral eye of about 2 weeks, about 3 weeks, 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, or more. In some embodiments, the individual had a calculated anti-VEGF (e.g., bevacizumab, brolocizumab, ranibizumab, and/or aflibercept) injection interval in the one eye and/or the contralateral eye of about 5-7 weeks, about 4-10 weeks, about 4-7 weeks, or about 4-6 weeks. In some embodiments, the individual received a prior treatment with an anti-VEGF agent (e.g., bevacizumab, brolocizumab, ranibizumab, faricimab, abicipar pegol, and/or aflibercept) in the one eye and/or in the contralateral eye any of at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, or at least about 20 days prior to administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye. In some embodiments, the individual received a prior treatment with an anti-VEGF agent (e.g., bevacizumab, brolocizumab, ranibizumab, faricimab, abicipar pegol, and/or aflibercept) in the one eye and/or the contralateral eye about 7 days, about 10 days, or about 14 days prior to administration

of the unit dose of rAAV particles to the one eye and/or the contralateral eye. In some embodiments, the prior treatment comprises an intraocular, subretinal or intravitreal injection with an anti-VEGF agent. In some embodiments, the anti-VEGF agent is bevacizumab, brolucizumab, ranibizumab, faricimab, abicipar pegol, and/or aflibercept. In some embodiments, the anti-VEGF agent is aflibercept.

[0206] In some embodiments, the individual demonstrated a meaningful response to a prior treatment with anti-VEGF agent. In some embodiments, the anti-VEGF agent is aflibercept, a functional variant thereof, or a functional fragment thereof. In some embodiments, the anti-VEGF agent comprises a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35. In some embodiments, the individual demonstrated a meaningful response to a prior anti-VEGF treatment (*e.g.*, aflibercept, a functional variant thereof, or a functional fragment thereof) for the ocular neovascular disease in the one eye and/or in the contralateral eye prior to administration of the unit dose of rAAV particles to one eye and/or the contralateral eye. In some embodiments, the individual is determined to have a meaningful response to a prior anti-VEGF treatment (*e.g.*, aflibercept, a functional variant thereof, or a functional fragment thereof) for the ocular neovascular disease if a reduction of $\geq 30\%$ (*e.g.*, any of at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100%) of central retinal thickness (CRT) or central subfield thickness (CST) is observed any of at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, or more after the anti-VEGF treatment, compared to the central retinal thickness (CRT) or central subfield thickness (CST) at the initial diagnosis in the one eye and/or the contralateral eye. In some embodiments, the individual is determined to have a meaningful response to a prior anti-VEGF treatment (*e.g.*, aflibercept, a functional variant thereof, or a functional fragment thereof) for the ocular neovascular disease if a reduction of $\geq 30\%$ (*e.g.*, any of at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100%) in central retinal thickness (CRT) or central subfield thickness (CST) is observed more than any of about 7 days, about 10 days, or about 14 days after the anti-VEGF treatment, compared to the central retinal thickness (CRT) or central subfield thickness (CST) prior to administration of the prior anti-VEGF treatment in the one eye and/or the contralateral eye.

[0207] In some embodiments, the central subfield thickness and/or central retinal thickness is determined by SD-OCT in the one eye and/or the contralateral eye. Central subfield thickness is the mean thickness of the retina across the central subfield of an ETDRS grid, a 1 mm diameter circle centered on the fovea.

[0208] In some embodiments, the individual is determined to have a meaningful response to a prior treatment with anti-VEGF agent (*e.g.*, aflibercept, a functional variant thereof, or a functional fragment thereof) for the ocular neovascular disease if a reduction of $\geq 20\%$ (*e.g.*, any of at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100%) in central subfield thickness and/or central retinal thickness is observed compared to the central subfield thickness

and/or central retinal thickness prior to administration of the prior treatment with the anti-VEGF agent. In some embodiments, the individual is determined to have a meaningful response to a prior treatment with anti-VEGF agent (*e.g.*, aflibercept, a functional variant thereof, or a functional fragment thereof) for the ocular neovascular disease if a reduction of $\geq 20\%$ (*e.g.*, any of at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100%) in central subfield thickness and/or central retinal thickness is observed any of at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, or more after administration of the prior treatment with an anti-VEGF agent, compared to the central subfield thickness and/or central retinal thickness prior to administration of the prior treatment with an anti-VEGF agent. In some embodiments, the individual is determined to have a meaningful response to a prior treatment with an anti-VEGF agent (*e.g.*, aflibercept, a functional variant thereof, or a functional fragment thereof) for the ocular neovascular disease if a reduction of $\geq 20\%$ (*e.g.*, any of at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100%) in central subfield thickness and/or central retinal thickness is observed about 7 days, about 10 days, or about 14 days after administration of the prior treatment with the anti-VEGF agent, compared to the central subfield thickness and/or central retinal thickness prior to administration of the prior treatment with an anti-VEGF agent. In some embodiments, the central subfield thickness and/or central retinal thickness is determined by SD-OCT in the one eye and/or the contralateral eye. In some embodiments, the individual is determined to have a meaningful response to a prior treatment with an anti-VEGF agent (*e.g.*, aflibercept, a functional variant thereof, or a functional fragment thereof) for the ocular neovascular disease if normalization of CST is observed with no observable vascular exudation after the anti-VEGF treatment in the one eye and/or the contralateral eye. Normalization refers to a CST value that is normal for that class of patient (*e.g.*, based on age, sex, etc.)

[0209] In some embodiments, the individual is determined to have a meaningful response to a prior anti-VEGF treatment (*e.g.*, aflibercept, a functional variant thereof, or a functional fragment thereof) for the ocular neovascular disease if a reduction of $\geq 20\%$ (*e.g.*, any of at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100%) in central retinal thickness (CRT) or central subfield thickness (CST) is observed any of at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, or more after the anti-VEGF treatment, compared to the central retinal thickness (CRT) or central subfield thickness (CST) prior to administration of the anti-VEGF treatment (*e.g.*, aflibercept), as determined by SD-OCT in the one eye and/or the contralateral eye. In some embodiments, the individual is determined to have a meaningful response to a prior anti-VEGF treatment (*e.g.*, aflibercept, a functional variant thereof, or a functional fragment thereof) for the ocular neovascular disease if a reduction of $\geq 20\%$ (*e.g.*, any of at least 20%, at least 30%, at least 40%, at least

50%, at least 60%, at least 70%, at least 80%, at least 90%, or 100%) in central retinal thickness (CRT) or central subfield thickness (CST) is observed about 7 days, about 10 days, or about 14 days after the anti-VEGF treatment, compared to the central retinal thickness (CRT) or central subfield thickness (CST) prior to administration of the anti-VEGF treatment, as determined by SD-OCT in the one eye and/or the contralateral eye.

[0210] In some embodiments, the individual has not received a prior treatment for an ocular neovascular disease. In some embodiments, the individual has not received a prior treatment in the one eye and/or the contralateral eye for an ocular neovascular disease. In some embodiments, the individual has not received a prior anti-VEGF treatment. In some embodiments, the individual has not received a prior anti-VEGF treatment in the one eye and/or the contralateral eye. In some embodiments, the individual has not received a prior aflibercept treatment. In some embodiments, the individual has not received a prior aflibercept treatment in the one eye and/or the contralateral eye.

[0211] In some embodiments, the ocular disease or disorder treated according to the methods described herein is diabetic macular edema. Diabetic macular edema (DME) is a swelling of the retina in diabetes mellitus due to leaking of fluid from blood vessels within the macula. The macula is the central portion of the retina, a small area rich in cones, the specialized nerve endings that detect color and upon which daytime vision depends. As macular edema develops, blurring occurs in the middle or just to the side of the central visual field. Visual loss from diabetic macular edema can progress over a period of months and make it impossible to focus clearly. Common symptoms of DME are blurry vision, floaters, double vision, and eventually blindness if it goes untreated. In some embodiments, methods and pharmaceutical compositions as disclosed herein are used to treat DME.

[0212] In some embodiments, the ocular disease or disorder treated according to the methods described herein is a retinal vein occlusion. Retinal vein occlusion is a blockage of the small veins that carry blood away from the retina. The retina is the layer of tissue at the back of the inner eye that converts light images to nerve signals and sends them to the brain. Retinal vein occlusion is most often caused by hardening of the arteries (atherosclerosis) and the formation of a blood clot. Blockage of smaller veins (branch veins or BRVO) in the retina often occurs in places where retinal arteries that have been thickened or hardened by atherosclerosis cross over and place pressure on a retinal vein. Symptoms of retinal vein occlusion can include a sudden blurring or vision loss in all or part of one eye.

[0213] In some embodiments, the ocular disease or disorder treated according to the methods described herein is choroidal neovascularization (CNV), also known as wet age-related macular degeneration (wAMD). Choroidal neovascularization can involve the growth of new blood vessels that originate from the choroid through a break in the Bruch membrane into the sub-retinal pigment epithelium (sub-RPE) or subretinal space, which can be a major cause of visual loss. CNV can create a sudden deterioration of central vision, noticeable within a few weeks. Other symptoms can include color disturbances, and metamorphopsia (distortions in which straight lines appears wavy). Hemorrhaging of the new blood

vessels can accelerate the onset of symptoms of CNV. CNV may also include feeling of pressure behind the eye.

[0214] The advanced “wet” form (neovascular or exudative) of AMD may frequently cause a rapid and often substantial loss of central vision in patients. In the wet form of AMD, choroidal neovascularization forms and develops into a network of vessels that may grow under and through the retinal pigment epithelium. As this is accompanied by leakage of plasma and/or hemorrhage into the subretinal space, there could be severe sudden loss of central vision if this occurs in the macula. The present disclosure contemplates treatment or prevention of AMD, wet AMD. In some embodiments, methods and pharmaceutical compositions as disclosed herein are used to treat AMD.

[0215] In some embodiments, methods described herein are used to prevent or treat an ocular disease or disorder in a subject who has received prior treatment with bevacizumab, brolocizumab, ranibizumab, faricimab, abicipar pegol, and/or aflibercept. In some embodiments, methods described herein are used to prevent or treat an ocular disease or disorder that is responsive to treatment with bevacizumab, brolocizumab, ranibizumab, and/or aflibercept.

[0216] In some embodiments, the individual was diagnosed with the ocular neovascular disease at least 1 day, at least 1 week, at least 1 month, at least 2 months, at least 4 months, at least 6 months, at least 12 months, at least 18 months, at least 24 months, at least 30 months, at least 36 months, at least 42 months, at least 48 months, at least 54 months, at least 60 months, at least 66 months, at least 72 months, at least 78 months, at least 84 months, at least 90 months, 96 months, at least 102 months, at least 108 months, at least 114 months, at least 120 months, at least 126 months, at least 132 months, or more prior to administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye.

[0217] The following description is presented to enable a person of ordinary skill in the art to make and use the various embodiments. Descriptions of specific devices, techniques, and applications are provided only as examples. Various modifications to the examples described herein will be readily apparent to those of ordinary skill in the art, and the general principles defined herein may be applied to other examples and applications without departing from the spirit and scope of the various embodiments. Thus, the various embodiments are not intended to be limited to the examples described herein and shown, but are to be accorded the scope consistent with the claims.

EXAMPLES

Example 1: An open label Phase 1 study of AAV2.7m8-aflibercept in neovascular (wet) age-related macular degeneration at a dose of 6×10^{11} vg/eye.

[0218] This example describes an open label Phase 1 study of AAV2.7m8-aflibercept, a rAAV vector containing the VEGF inhibitor aflibercept and the AAV2.7m8 protein capsid, for the treatment of age-related macular degeneration (AMD) with choroidal neovascularization (wet AMD; wAMD).

I. Study Objectives

A. Primary Objective

[0219] The primary objective of this study was to assess the safety and tolerability of a single intravitreal (IVT) injection of AAV2.7m8-aflibercept in subjects with wAMD.

Primary Endpoints

[0220] The primary endpoints of this study were the type, severity, and incidence of ocular and systemic adverse events (AEs).

B. Secondary Objectives

[0221] The secondary objectives of this study were:

- To evaluate the effect of AAV2.7m8-aflibercept on Best Corrected Visual Acuity (BCVA).
- To evaluate the effect of AAV2.7m8-aflibercept on central subfield thickness (CST), also known as central retinal thickness (CRT).
- To assess the need for rescue therapy from Week 4 to Week 104.
- To evaluate the effect of AAV2.7m8-aflibercept on the presence of intraretinal fluid (IRF) and subretinal fluid (SRF).
- To evaluate the effect of AAV2.7m8-aflibercept on pigment epithelial detachment (PED) resolution among patients with a PED at baseline.

Secondary Endpoints

[0222] The secondary endpoints of this study were:

- The mean change in BCVA from baseline over time, as assessed by ETDRS letters over time from Day 8 to Week 104, compared to baseline.
- The percentage of subjects with a BCVA gain of ≥ 15 ETDRS letters from baseline over time, as assessed from Day 8 to Week 104, compared to baseline.
- The percentage of subjects with a BCVA decline of ≤ 15 ETDRS letters from baseline over time, as assessed from Day 8 to Week 104, compared to baseline.
- The mean change in CST and macular volume from Baseline over time, as assessed from Day 8 to Week 104, compared to baseline.
- The mean number of aflibercept injections over time, as assessed from Weeks 4-104.
- The percentage of subjects requiring aflibercept injections over time, as assessed from Weeks 4- 104.

- The percentage of subjects without IRF over time, as assessed from Day 8 to Week 104.
- The percentage of subjects without SRF over time, as assessed from Day 8 to Week 104.
- The percentage of subjects without a PED over time, as assessed from Day 8 to Week 104 among subjects with a PED at baseline.

II. Study Subjects

[0223] Subjects in this study were diagnosed with active choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD), a history of recent responsiveness to anti-VEGF treatment, and required frequent injections of anti-VEGF therapy.

[0224] Only one eye was selected as the study eye for the duration of the study. If both eyes met all of the inclusion and exclusion criteria, the eye with the worst BCVA assessed at Screening was selected as the study eye. If both eyes met all of the inclusion and exclusion criteria and BCVA values were identical for both eyes, the subject chose to select his/her non-dominant eye for treatment, or by default the right eye was selected as the study eye.

A. Inclusion Criteria

[0225] Subjects that met the following inclusion criteria were enrolled in this study:

- Male or female subjects, age ≥ 50 years of age.
- The study eye had prior or current evidence of active subfoveal CNV secondary to AMD occupying $\geq 50\%$ of total lesion size with:
 - Leakage on fluorescein angiogram (FA), fluid on Spectral Domain Optical Coherence Tomography (SD-OCT), or subretinal hemorrhage on color fundus photo; and
 - The entire dimension of the lesion did not exceed 12 Macular Photocoagulation Study disc areas.
- Subjects were under active anti-VEGF treatment for wAMD with a minimum of 2 injections within 4 months prior to Screening.
- Vision of study eye at Screening visit (prior to aflibercept injection):
 - BCVA ETDRS of 78-25.
- Vision of non-study eye:
 - BCVA ETDRS of ≥ 35 .
- Demonstrated a meaningful anti-VEGF response as confirmed by the Investigator and defined as:
 - Reduction from initial diagnosis in central subfield thickness by $\geq 30\%$ as assessed using SD-OCT; or
 - Reduction from screening in central subfield thickness by $\geq 20\%$ as assessed using SD-OCT; or

- Normalization of CST with no observable vascular exudation.

[0226] VEGF responsiveness was confirmed by the Investigator for purposes of confirmation of anti-VEGF response at Day 1 visit prior to dosing with AAV2.7m8-aflibercept. Subjects determined not to have a meaningful anti-VEGF response failed screening and were not enrolled in this study.

B. Exclusion Criteria

[0227] Subjects that met the following exclusion criteria were not enrolled in this study:

Neutralizing Antibodies

- Although there is not an established correlation between levels of neutralizing antibodies (NABs) to AAVs in serum and vitreous fluid (Lukason *et al.*, (2011) Mol Ther 19(2):260-265), subjects were screened for NABs to the AAV2.7m8 vector as a precaution. Subjects with documented anti-AAV2.7m8 neutralizing antibody titer levels > 1:125 within 6 months prior to dosing with AAV2.7m8-aflibercept were excluded from this study.

CNV Lesion

- Known history or evidence of the following CNV lesion characteristics:
 - Fibrosis or atrophy, retinal epithelial tear in the center of the fovea in the study eye, or any condition preventing visual acuity improvement.
 - Scarring or fibrosis making up > 50% of total lesion area.
 - Lesion size > 12 Macular Photocoagulation Study disc areas (30.5 mm²), including blood, scars, and neovascularization as assessed by Fluorescein Angiography (FA).
 - Subretinal hemorrhage that was ≥ 50% of the total lesion area, or the presence of blood under the fovea that was ≥ 1 disc area in size in the study eye (if blood was under the fovea, then the fovea was surrounded 270 degrees by visible CNV).

Ocular Conditions (Retina/Posterior Eye)

- Significant epiretinal membrane or vitreomacular traction (VMT) syndrome in the study eye at time of dosing with AAV2.7m8-aflibercept or history of a full thickness macular hole (Gass Stage 2 and above) in the study eye.
- History of retinal disease in the study eye other than wAMD, including diabetic retinopathy (in either eye), retinal vein occlusion, uveitis, suspected retinal angiomatous proliferation, polypoidal choroidopathy, or CNV due to other causes (*e.g.*, ocular histoplasmosis, trauma, or pathologic myopia), or any other vascular disease in the eye (benign conditions of the vitreous or peripheral retina were non-exclusionary).
- History of retinal detachment (with or without repair) in the study eye.

Other Conditions (Non-Retinal)

- Known history or evidence of significant non-retinal disease or media opacity in the study eye that could have compromised vision during the course of the study, required surgery and/or

precluded proper visualization or imaging of the retina (*e.g.*, central corneal scarring, significant cataract, corneal dystrophy, scleromalacia).

- Uncontrolled ocular hypertension or glaucoma in the study eye at time of dosing with AAV2.7m8-aflibercept (defined as intraocular pressure [IOP] > 22 mmHg despite treatment with anti-glaucoma medication) or use of > 2 IOP lowering medications at the time of screening.
- Active or history of ocular or periocular infection in either eye within 4 weeks prior to dosing with AAV2.7m8-aflibercept.

Ocular Surgeries/Procedures

- Any previous intraocular or periocular surgery on the study eye within 6 months of dosing with AAV2.7m8-aflibercept, or any planned major surgical procedure within 6 months of dosing with AAV2.7m8-aflibercept. Lid surgery > 1 month of dosing with AAV2.7m8-aflibercept was not an exclusion criterion.
- History of vitrectomy, trabeculectomy, or other filtration surgery in the study eye.
- Yttrium aluminum garnet (YAG) posterior capsulotomy within 3 months prior to dosing with AAV2.7m8-aflibercept.
- Any prior treatment with photodynamic therapy or retinal laser for the treatment of wAMD and any previous therapeutic radiation in the region of the study eye.

General/Systemic Conditions

- History or evidence of any of the following cardiovascular diseases within 6 months of dosing unless specified:
 - Severe cardiac disease (*e.g.*, New York Heart Association [NYHA] Functional Class III or IV) history or clinical evidence of unstable angina.
 - Acute coronary syndrome, myocardial infarction or coronary artery revascularization.
 - Ventricular tachyarrhythmias requiring ongoing treatment, or uncontrolled arrhythmia.
 - Uncontrolled hypertension defined as average systolic blood pressure (SBP) ≥ 160 mmHg or an average diastolic blood pressure (DBP) ≥ 100 mmHg, despite using BP-lowering medication within the screening period prior to dosing.
 - History of cerebrovascular accident or transient ischemic attack.
- Any history of ongoing bleeding disorders or international normalized ratio (INR) >3.0. The use of aspirin or other anticoagulants (*e.g.*, Factor Xa inhibitors) was not an exclusion criterion. INR was repeated during the Screening period to confirm eligibility criteria were met.
- Evidence of uncontrolled diabetes with an HbA1c >7.0% within the screening period prior to dosing with AAV2.7m8-aflibercept.
- History of malignancy within the last 5 years except for the following, if adequately treated:

- Local basal cell or squamous cell carcinoma of the skin.
- Carcinoma *in situ* of the cervix or breast.
- Papillary, noninvasive bladder cancer.
- Prostate cancer Stage 1 and 2 for which observation was clinically indicated with stable prostate-specific antigen (PSA) for 6 months.
- Any other cancer that was in complete remission for at least 2 years or considered surgically cured.
- Positive HIV, Hepatitis B, or Hepatitis C (unless treated with a documented cure).
- Within 36 hours prior to dosing with AAV2.7m8-aflibercept, evidence or suspicion of systemic active infection of any type deemed clinically significant by the Investigator based on clinical exam and/or temperature $>38.5^{\circ}\text{C}$.
- Known serious allergies to:
 - Fluorescein dye or sodium fluorescein used in angiography (mild allergy amenable to treatment was allowable); or
 - Aflibercept.
- Women who were pregnant, breastfeeding, or intend to become pregnant during the study.
- Other significant laboratory abnormalities or medical conditions that compromised the subject's safety in the view of the Investigator.

Medications

- Use of systemic anti-inflammatory steroids or immunosuppressant medications (other than protocol-specified prednisone) within 5 half-lives prior to dosing with AAV2.7m8-aflibercept. Inhaled or topical steroids and nonsteroidal anti-inflammatory drugs (NSAIDs) were permitted.
- Received any of the following:
 - Investigational medicinal product within 30 days or 5 half-lives prior to dosing with AAV2.7m8-aflibercept, whichever was longer.
 - Prior gene therapy.

III. Investigational Medicinal Product

[0228] The investigational medicinal product (IMP), AAV2.7m8-aflibercept, was a recombinant, replication-deficient adeno-associated viral (rAAV) vector containing the AAV2.7m8 protein capsid derived by *in vivo* directed evolution on the AAV2 capsid (Dalkara *et al.*, (2013) Sci Transl Med 5(189):189ra76; US2014/0364338). AAV2.7m8-aflibercept carried an expression cassette of a codon-optimized version of the aflibercept cDNA under the control of a ubiquitous chimeric promoter (FIG. 1A) (See WO2018170473A1). AAV2.7m8-aflibercept was manufactured using a baculovirus expression vector system in Sf9 cells where two different baculoviruses were used, one encoding the genes for AAV2

Rep and AAV2.7m8 Cap proteins, and the other encoding the human aflibercept cDNA expression cassette.

[0229] AAV2.7m8-aflibercept was supplied as a sterile-filtered, frozen suspension in a sterile, ready-to-use 0.5 mL Crystal Zenith vial which contained 0.25 mL of IMP, formulated as shown in Table 1.

Table 1: Formulation of the AAV2.7m8-aflibercept investigational medicinal product for Cohort 1.

Formulation (pH 7.3)		
Components	Concentration	Function
AAV2.7m8-aflibercept	6×10^{12} vg/mL	Active ingredient
Sodium Chloride	180 mM	Osmotic/ionic strength agent
Sodium Phosphate Monobasic	5 mM	Buffering agent
Sodium Phosphate Dibasic	5 mM	Buffering agent
Poloxamer 188	0.001% (w/v)	Surfactant
Water for Injection	Quantum satis (Q.S.)	Solvent
Injection Volume	100 μ L	

IV. Study Design

A. Dose and Method of Administration

Cohort 1

[0230] Subjects in Cohort 1 were administered a single IVT injection of AAV2.7m8-aflibercept at Dose 1 of 6×10^{11} vg/eye in an injection volume of 100 μ L.

[0231] AAV2.7m8-aflibercept vials were removed from the frozen storage at $\leq -60^{\circ}\text{C}$ and thawed at room temperature. AAV2.7m8-aflibercept was administered via IVT injection. Aseptic technique with povidone-iodine was employed, along with topical or subconjunctival anesthesia. Post-injection care and medication regimen were given based on institutional standard of care.

B. Study Visits

Screening (Days -15 to -7)

[0232] As shown in FIG. 1B, subjects received a single IVT injection of aflibercept 2 mg during Screening on Days -15 to -7 (e.g., Days -14- to -7), consistent with standard of care. Subjects received routine institutional standard post-injection care.

Study Day 1

[0233] On Study Day 1 (between 7 and 15 days, e.g., between 7 and 14 days, after the IVT injection of aflibercept), study subjects underwent SD-OCT studies to confirm that they were responsive to anti-VEGF therapy, prior to dosing with AAV2.7m8-aflibercept. Anti-VEGF responsiveness was confirmed

by the Investigator. Only subjects confirmed to have a meaningful anti-VEGF response, as described above (*See Inclusion Criteria*), were eligible to enroll in this study.

[0234] Subjects responsive to anti-VEGF therapy were sequentially enrolled into the study cohort and received a single AAV2.7m8-aflibercept IVT injection in the study eye. As described above, only one eye was selected as the study eye for the duration of the study.

Post-AAV2.7m8-aflibercept Administration

[0235] Subjects returned on Days 3 and 8, during Weeks 2, 4, 6, and 8, and every 4 weeks thereafter (*i.e.*, weeks 12, 16, 20, and 24) for clinical evaluation and treatment (if necessary). A safety and efficacy analysis of Cohort 1 was performed at week 24.

[0236] Starting on Week 4, subjects were eligible to receive rescue injections of aflibercept 2 mg IVT if there was evidence of increased disease activity according to the retreatment criteria (see below). The presence of any one of the following warranted resumption of standard anti-VEGF treatment with 2mg IVT aflibercept:

- Loss of ≥ 10 letters in BCVA (using the ETDRS protocol) from Baseline and intraretinal or subretinal fluid observed by SD-OCT and judged by the Investigator to be the cause of the BCVA loss.
- An increase in central subfield thickness $> 75 \mu\text{m}$ from Baseline as assessed by SD-OCT.
- Presence of vision-threatening hemorrhage due to macular degeneration.

[0237] Subjects are evaluated again on week 52 following administration of AAV2.7m8-aflibercept for safety and efficacy. The follow up period continues until week 104 following administration of AAV2.7m8-aflibercept.

C. Corticosteroid Regimen

[0238] To reduce the possible risk of post-injection ocular inflammation, subjects were administered a prophylactic corticosteroid regimen (*e.g.*, prednisone) and monitored closely for ocular and systemic tolerability of the vector.

[0239] Subjects in Cohort 1 were administered a prophylactic 13-day oral corticosteroid regimen, starting with 60 mg/day of prednisone from 3 days before (day -3) to 3 days after AAV2.7m8-aflibercept treatment for a total of 6 days. This was followed by a 7-day prednisone taper. A summary of the oral prednisone regimen is provided in Table 2.

Table 2: Oral prednisone regimen.

Study Days	Total Number of Days	Prednisone Dose per Day
Days -3 to +3	6	60 mg
Days 4-6	3	40 mg
Days 7-8	2	20 mg
Days 9-10	2	10 mg
Day 11	0	STOP

[0240] Initiating immunosuppression (*i.e.*, prednisone) prior to AAV2.7m8-aflibercept IVT injection was designed to limit the immune response upon exposure to capsid antigens. Subjects self-administered prednisone for the 13-day regimen.

[0241] Subjects received topical or oral corticosteroids (prednisone) as needed during weeks 2-24 of the study.

D. Prohibited Medications and Treatments

[0242] The following medications were prohibited during the study:

- Any systemic anti-VEGF agent including bevacizumab.
- Any anti-VEGF agent in the study eye other than the study drug or aflibercept injection 2 mg, according to the rescue anti-VEGF injection criteria in this study.
- IVT steroids in the study eye.
- Immune suppression drugs. Systemic, inhaled or topical steroids and NSAIDs were allowed.
- Use of and participation in any other investigational studies.
- Cataract Surgery in the study eye could be performed if clinically indicated and was scheduled > 90 days after IVT administration and/or >7 days after the last injection of aflibercept.
- Subjects who developed AMD in the non-study eye could receive standard of care therapy in the non-study eye.

E. Summary of Study Design for Cohort 1

[0243] Six subjects were enrolled into Cohort 1.

[0244] Subjects in Cohort 1 were administered a single IVT injection of AAV2.7m8-aflibercept at Dose 1 of 6×10^{11} vg/eye. The first (sentinel) subject enrolled in Cohort 1 received an IVT injection of

AAV2.7m8-aflibercept and was evaluated for 29 days prior to dosing the subsequent 5 subjects (Subjects 2-6) within the cohort.

[0245] A summary of the study design for Cohort 1 is provided in Table 3.

Table 3: Summary of study design for Cohort 1.

Cohort	AAV2.7m8-aflibercept Dose	Corticosteroid Regimen
Cohort 1	6×10^{11} vg/eye	Prophylactic oral prednisone regimen.

F. Study Duration

[0246] Duration of subject participation in the study is approximately 108 weeks for each subject. This includes a screening period of 4 weeks and an additional 104-week study period.

[0247] Upon completion or discontinuation of the study, if appropriate, subjects are offered the opportunity to enroll in a long-term follow-up study to further assess the safety of this gene therapy.

V. Study Assessments

A. General Physical Examination and Vital Signs

[0248] Each subject's relevant medical and ophthalmic history was collected and recorded. A general physical examination consisted of height (at Screening only), body weight, and vital signs.

[0249] Vital signs consisted of blood pressure, pulse rate measurements, body temperature, and respiratory rate. A 12-lead electrocardiogram (ECG) was taken for each subject. The following clinical laboratory and antibody tests were performed: chemistry, complete blood count (CBC), coagulation studies, urinalysis, serological evidence of HIV or Hepatitis, and pregnancy testing.

[0250] At the Week 104, end of study (EOS), and/or Early Termination visit, a physical examination assesses if any changes in the subject's physical condition occurred since the Screening examination.

B. Immune Response and Aflibercept Expression

[0251] Total anti-AAV2.7m8 antibodies were measured. Neutralizing anti-AAV2.7m8 antibodies in subject's serum were determined using a reporter gene-based transduction interference assay assessed by cutpoint.

[0252] The humoral immune response against anti-aflibercept antibodies was measured in serum using an ELISA-based cutpoint antibody assay.

[0253] Serum was collected to determine the presence of aflibercept protein.

[0254] Cellular immunity against AAV2.7m8 capsid proteins and aflibercept protein were measured using an ELISPOT assay.

C. Full Ophthalmic Examination and Other Assessment Methods

[0255] Study assessments included an ophthalmologic exam, intraocular pressure (IOP), and indirect ophthalmoscopy.

[0256] The ophthalmic examination consisted of an external examination of the eye and adnexa, routine screening for eyelid/pupil responsiveness (including but not limited to blepharoptosis, abnormal pupil shape, unequal pupils, abnormal reaction to light, and afferent pupillary defect), and slit-lamp examination (eyelids, conjunctiva, cornea, lens, iris, anterior chamber). The slit-lamp examination examined the anterior ocular structures and was used for grading any findings. If any finding was noted during the slit-lamp examination, at any visit, the severity was graded by the Investigator and the finding was described as clinically significant or not clinically significant.

[0257] The IOP measurements were performed using a Goldmann applanation tonometer or Tono-pen™. IOP measurements were performed prior to any IVT injection and prior to dilating eyes. Day 1 visit required pre-injection and post-injection (30 minutes after injection) IOP measurements.

[0258] The dilated indirect ophthalmoscopy examination included an evaluation of posterior segment abnormalities of the vitreous, optic nerve, peripheral retina, and retinal vasculature. If any finding was noted during the ophthalmoscopy, at any visit, the severity was graded by the Investigator and the finding was described as clinically significant or not clinically significant. Day 1 visit required pre-injection and post-injection indirect ophthalmoscopy assessments.

Spectral Domain Optical Coherence Tomography (SD-OCT)

[0259] SD-OCT was used to obtain depth-resolved tissue structure information encoded in the magnitude and delay of the back-scattered light by spectral analysis of the interference fringe pattern.

Fluorescein Angiography

[0260] Fluorescein angiography images were used to confirm patient eligibility for study enrollment, to assess the efficacy of CNV lesion growth, and to evaluate leakage compared to Baseline.

Digital Color Fundus Photography

[0261] Color fundus images of the retina, optic disc, and macula were taken.

Optical Coherence Tomography Angiography (OCT-A)

[0262] OCT-A imaging (swept-source or spectral-domain) was used to obtain volumetric, three-dimensional maps of the retina and choroid as well as information on blood flow.

Refraction and Visual Acuity

[0263] Refraction and BCVA were measured by a trained and certified visual acuity examiner at the study sites. Visual acuity was measured at a starting distance of 4 meters, prior to dilating eyes.

D. Safety Assessments

[0264] To mitigate the risks associated with the IVT administration of AAV2.7m8-aflibercept, subjects were closely monitored on the day of the IVT AAV2.7m8-aflibercept administration and thereafter post-treatment.

[0265] The safety of AAV2.7m8-aflibercept was assessed through the collection of AEs, vital signs, physical and eye examinations, ECG, pregnancy testing, and laboratory evaluations.

[0266] Intense monitoring of subjects was done in the first 8 weeks of the study, followed by regular safety assessments for safety and efficacy thereafter. All subjects had their visual acuity tested by Early Treatment Diabetic Retinopathy Study (ETDRS) letters assessments at each study visit, and standard of care aflibercept IVT injections were used as a rescue treatment.

[0267] The severity, or intensity, of an AE was rated on the following scale:

- Mild: the AE was noticeable but did not significantly impair the subject's daily activities.
- Moderate: the AE reduced or impaired normal daily activity but was not incapacitating.
- Severe: the AE was incapacitating and resulted in an inability to perform normal daily activity.

[0268] Safety is assessed over 104 weeks post-administration of study treatment. Upon completion of the End of Study (EOS) Visit, subjects are offered enrollment in a long-term extension study to further assess the safety and durability of transgene expression.

E. Efficacy Assessments

[0269] The efficacy of AAV2.7m8-aflibercept in the treatment of wAMD was assessed by the following measures. A key assessment time point was at 24 weeks. The baseline values of BCVA and SD-OCT refer to the values taken during the screening visit on Days -15 to -7 (e.g., Days -14 to -7) prior to aflibercept injection. The baseline values were used to compare for analysis.

[0270] Vision was assessed primarily through the BCVA expressed as an ETDRS score (number of letters correctly read) (Vitale *et al.*, (2016) JAMA Ophthalmol 134(9):1041-1047). Maintenance of vision was classified if the subject lost fewer than 15 letters in the ETDRS score compared to Baseline. Calculated endpoints included the mean change from Baseline, the percent gaining at least 15 letters compared to Baseline, and the percent losing 15 or more letters compared to Baseline.

[0271] FA was performed to assess CNV lesions using a standard technique to evaluate leakage compared to Baseline.

[0272] SD-OCT was performed using approved equipment and standard techniques to evaluate retinal thickness (e.g., central retinal thickness or central subfield thickness), macular volume, and the presence of fluid (e.g., subretinal fluid and intraretinal fluid) compared to baseline values.

[0273] The number of aflibercept injections given post AAV2.7m8-aflibercept treatment per subject, over time, from Week 4 to Week 104 were determined. In addition, the time to the first aflibercept injection post treatment with AAV2.7m8-aflibercept and the proportion of subjects who did not require an aflibercept rescue treatment were determined.

[0274] The proportion of subjects without IRF over time from week 4 to week 104 was determined.

[0275] The proportion of subjects without SRF over time from week 4 to week 104 was determined.

[0276] The proportion of subjects without a PED over time from week 4 to week 104 among subjects with a PED at baseline was determined.

F. Statistics

[0277] The Safety population included all subjects who received AAV2.7m8-aflibercept and were analyzed according to the dose received.

[0278] All other safety parameters were summarized by cohort. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA, version 21) classification to give a preferred term (PT) and system organ class (SOC) for each event. SAEs and AEs leading to study withdrawal were listed separately.

[0279] Efficacy analyses included all subjects. Efficacy analysis endpoints were evaluated, and descriptive statistics will be calculated by cohort. The key assessment time point was at 24 weeks. Efficacy was assessed according to dose received and in aggregate.

VI. Results

A. Subject Characteristics

[0280] All six subjects enrolled in Cohort 1 were diagnosed wAMD. At the time of enrollment, subjects had a high requirement for anti-VEGF treatment (e.g., required frequent anti-VEGF treatment), functional vision around 20/50, some excess central subfield thickness on OCT, and were undergoing regular IVT injections of anti-VEGF treatment and responding to therapy. Disease characteristics and treatment histories for all subjects are provided in Tables 4-5.

[0281] As shown in Table 4, subjects in Cohort 1 were diagnosed with wAMD between approximately one year (Subject 5) to approximately a decade or more (Subject 4) prior to being administered AAV2.7m8-aflibercept. Subjects had received a wide range of prior anti-VEGF IVT injections in the study eye, from 7 (Subject 5) to 109 (Subject 4) prior injections. The calculated average anti-VEGF IVT injection interval prior to this study ranged from every 4 weeks to every 10 weeks. All subjects received 2 or 3 anti-VEGF injections in the four months prior to screening for enrollment in this study. Subjects were

administered AAV2.7m8-aflibercept 7 days (Subjects 1-3) or 14 days (Subjects 4-6) after the Screening anti-VEGF injection prior to the start of the study.

Table 4: Baseline characteristics of subjects in Cohort 1.

Characteristic	Value
Mean age, years	79.0
Mean time since nAMD diagnosis, years	3.3
Mean number anti-VEGF injections since initial diagnosis (range)	35.3 (7–109)
Mean number of anti-VEGF injections in 4 months prior to screening	2.8
Mean number anti-VEGF injections in 8 months prior to screening	6.2
Average annualized injection frequency	9.3
Baseline BCVA in study eye (ETDRS letters), mean	65.8
Approximate Snellen equivalent	20/50
Baseline mean CST study eye, μm	369.2

Table 5: Disease characteristics and treatment histories of study subjects in Cohort 1.

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
Age	76 years	83 years	87 years	62 years	88 years	77 years
Gender	Male	Male	Male	Male	Male	Female
Time between neovascular AMD diagnosis and AAV2.7m8-aflibercept IVT injection:	2.3 years	1.1 years	1.2 years	11.0 years	1.0 years	7.0 years
Number of prior anti-VEGF injections in study eye:	18	9	29	109	7	8

Calculated anti-VEGF injection interval average pre-study:	5-7 weeks	4-10 weeks	4-7 weeks	4-6 weeks	4-7 weeks	4-6 weeks
Number of anti-VEGF injections received in 4 months prior to screening:	3	2	3	3	2	4
Number of anti-VEGF injections received in 8 months prior to screening:	5	6	6	6	6	8
Average annualized injection frequency	7.5	9	9	9	9	12
Days between Screening aflibercept IVT injection and AAV2.7m8-aflibercept IVT injection:	7 days	7 days	7 days	14 days	14 days	10 days

B. Safety

[0282] During the 24 weeks following administration of AAV2.7m8-aflibercept, no SAEs occurred, nor were there any AEs that met the criteria for dose limiting toxicity (DLT). No drug-related non-ocular AEs were observed. Ocular inflammation was observed in all subjects, and was manageable with topical steroids. In addition, no vasculitis, retinitis, or choroiditis were observed. Nineteen ocular AEs were observed that were deemed potentially related to AAV2.7m8-aflibercept, 14 were mild, and 5 were moderate (2 AEs were intermediate uveitis, 1 AE was vitreous cells, 2 AEs were anterior chamber cells). One patient had two anterior chamber cell events (one mild event and one moderate event). Mild-to-moderate intraocular inflammation, responsive to topical or oral corticosteroids, was frequently observed during early follow-up. OCT images showed resolution of persistent fluid in most subjects, with no signs of worsening. Visual acuity was generally stable. A summary of all safety events that were related to AAV2.7m8-aflibercept through 24 weeks is provided in Table 6.

Table 6: All safety events related to AAV2.7m8-aflibercept.

AAV2.7m8-aflibercept-related ocular AEs	No. of Patients, n	Number of Events, n						
		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Total
Anterior Chamber Cells	3	0	1	0	1*	0	2 [†]	4
AC Flare	3	1	0	2	0	1	0	4
Vitreous Cells	3	0	1*	1	0	0	1	3
Intermediate Uveitis	2	0	1*	0	0	0	1*	2
Keratic Precipitates	1	0	0	2	0	0	0	2
Poor Pupil Dilation	1	0	0	0	0	1	0	1
Ocular Floaters	1	0	1	0	0	0	0	1
Vitreous Debris	1	0	0	0	0	0	1	1
Vitreous Haze	1	0	0	1	0	0	0	1
* Moderate Event; All events were either mild (14) or moderate (5) in intensity. [†] Patient had two anterior chamber cell events (one mild event and one moderate event).								

[0283] All subjects received 60mg of oral prednisone for 6 days starting at day -3 followed by a 7-day tapering course of prednisone. Clinical assessment of ocular cellular inflammation revealed that no clinically significant inflammation occurred early post-AAV2.7m8-aflibercept. In addition, as shown in **FIG. 6**, no worsening or new inflammation was observed when subjects were receiving steroid eye drops. By week 24, anterior chamber cellular inflammation was resolved or improving. Observed cellular inflammation was generally mild. The aqueous cell count categories shown in **FIG. 6** were based on the Standardization of Uveitis Nomenclature (SUN) criteria (Jabs, DA *et al.*, J Ophthalmol. 2005;140:509–516), whereas vitreous cell count categories were based on National Institutes of Health (NIH) guidelines.

[0284] Overall, safety assessments showed that AAV2.7m8-aflibercept was well tolerated, with no DLTs or SAEs reported. All safety events were mild to moderate, and most were inflammation-related. Continued follow-up of AEs showed that mean visual acuity remained stable and no anti-VEGF rescue injections were required. Thus, the results indicate that AAV2.7m8-aflibercept administered as a single

IVT injection at a dose of 6×10^{11} vg/eye in wAMD patients previously requiring frequent anti-VEGF injections has an acceptable safety profile.

C. Efficacy

[0285] Following AAV2.7m8-aflibercept administration, all six subjects showed stabilization of disease activity based on OCT assessments. As shown in FIGS. 2A-2L, OCT images taken prior to and after treatment with AAV2.7m8-aflibercept indicated that a robust anatomical response was evident in all six subjects in Cohort 1. For example, subretinal fluid persisted with standard of care anti-VEGF treatment, but resolved and remained resolved following administration of AAV2.7m8-aflibercept in Subject 1 (e.g., compare FIGS. 2A and 2B), Subject 2 (e.g., compare FIGS. 2B and 2C), Subject 3 (e.g., compare FIGS. 2D and 2E), Subject 4 (e.g., compare FIGS. 2F and 2G), and Subject 5 (e.g., compare FIGS. 2H and 2I). Subject 6 exhibited retinal morphology consistent with polypoidal choroidal vasculopathy (PCV) (FIG. 2K), and while some fluid persisted after treatment with AAV2.7m8-aflibercept, there was no evidence of disease progression (FIG. 2L). In addition, as shown in FIG. 3, none of the subjects exhibited an increase in central subfield thickness (CST) and a mean decrease of $-52.7 \mu\text{m}$ was observed (90% CI - 86.5, -18.8). Increased CST is indicative of disease progression in wAMD. Importantly, none of the subjects were administered or required any standard of care aflibercept rescue injections ("rescue injections").

[0286] Best corrected visual acuity (BCVA) was measured based on ETDRS letters assessments in all subjects throughout the study. As shown in FIG. 4, BCVA was stable across subjects in Cohort 1 with a mean decrease of -2 letters (90% CI -9.1, 5.1).

[0287] At a median follow up time of 34 weeks after administration of AAV2.7m8-aflibercept, no subjects exhibited signs of disease reactivation on OCT imaging (FIGS. 7A-7B). In addition, no subjects required any rescue anti-VEGF IVT injections and no subjects met retreatment criteria at any point during the follow-up period of up to 44 weeks. Finally, during the additional follow-up period beyond 24 weeks, BCVA was maintained (i.e., no patient lost or gained greater than 10 ETDRS letters), the anatomic improvements (i.e., resolution of subretinal and intraretinal fluid, and reductions in CST) observed at week 24 were maintained, and no safety concerns arose.

[0288] A summary of safety and efficacy results evaluated at the median follow up time of 34 weeks is provided in Table 7.

Table 7: Summary of safety and efficacy results at median follow up time of 34 weeks.

	Value
Median follow-up, weeks	34.0
Follow-up (min, max), weeks	28, 44
Grade 3 adverse events, n	0
Serious adverse events, n	0
Dose-limiting toxicities, n	0
Mean BCVA change from baseline, ETDRS letters	-1.5
BCVA change from baseline (min, max), ETDRS letters	-9, +5
Total number of rescue injections, n	0

D. Conclusions

[0289] The current standard of care therapy for wAMD is anti-VEGF IVT injections that are typically required long term, about every 4-8 weeks. Compliance with this regimen can be difficult for patients, caregivers, and healthcare systems, leading to suboptimal dosing and loss of vision due to undertreatment.

[0290] The results presented in this example show that AAV2.7m8-aflibercept provided a clear benefit for improving retinal anatomy and stabilizing vision, while exhibiting an acceptable safety profile. Specifically, patients maintained vision during the study, and AAV2.7m8-aflibercept was shown to be safe and well tolerated, with observed inflammation generally being mild and responsive to steroid eye drops.

[0291] It was noted that subjects in Cohort 1 of this study previously required frequent anti-VEGF injections (Table 4) in order to slow or prevent progression of their disease, however no anti-VEGF rescue injections were required during this study following administration of AAV2.7m8-aflibercept.

[0292] It was noted that subretinal fluid was present on OCT scans of subjects undergoing standard of care anti-VEGF treatment for more than 20 weeks, and continued 1-2 weeks after the Screening aflibercept IVT injection. This fluid was present even though subjects had been treated repeatedly and was thus refractory to standard of care IVT bolus of anti-VEGF protein. Unexpectedly, this refractory SRF resolved after treatment with AAV2.7m8-aflibercept, a result which would not have been predicted from preclinical studies.

[0293] The observed safety and efficacy of AAV2.7m8-aflibercept administered at a dose of 6×10^{11} vg/eye led to the continuation of this Phase 1 study to assess the safety and efficacy of AAV2.7m8-aflibercept administered at lower doses and with topical corticosteroids, as described in Example 2.

Example 2: An open label Phase 1 study of AAV2.7m8-aflibercept in neovascular (wet) age-related macular degeneration at doses lower than 6×10^{11} vg/eye and with topical corticosteroids.

[0294] The following example describes a continuation of the Phase 1 study described in Example 1 to assess the safety and efficacy of AAV2.7m8-aflibercept administered at doses lower than 6×10^{11} vg/eye and with topical corticosteroids in subjects with wAMD.

I. Study Objectives and Endpoints

[0295] The primary objective, secondary objectives, and primary and secondary endpoints are as described in Section I of Example 1.

II. Study Subjects

[0296] The study subjects in this study are as described in Section II of Example 1.

III. Investigational Medicinal Product

[0297] The investigational medicinal product is AAV2.7m8-aflibercept, as described in detail in Section III of Example 1, and as depicted in FIG. 1A with the exception that the concentration of AAV2.7m8-aflibercept was varied in order to maintain a suitable injection volume as described in Table 8, below.

Table 8: Formulation of the AAV2.7m8-aflibercept investigational medicinal product for cohorts 2–4.

	AAV2.7m8-aflibercept Concentration	Injection Volume
Cohort 2	6×10^{12} vg/mL	30 μ L
Cohort 3	6×10^{12} vg/mL	30 μ L
Cohort 4a	6×10^{12} vg/mL	100 μ L
Cohort 4b	6×10^{11} vg/mL	100 μ L

IV. Study Design

A. Dose and Method of Administration

[0298] AAV2.7m8-aflibercept at the doses described below was administered as described in Section IV of Example 1.

Study Cohorts 2-4

[0299] **Cohort 2:** Six subjects who are diagnosed with wAMD are enrolled into Cohort 2. Subjects in Cohort 2 are administered a single IVT injection of AAV2.7m8-aflibercept at Dose 2 of 2×10^{11} vg/eye with a prophylactic oral prednisone regimen.

[0300] **Cohort 3:** Nine subjects who are diagnosed with wAMD are enrolled into Cohort 3. Subjects in Cohort 3 are administered a single IVT injection of AAV2.7m8-aflibercept at Dose 2 of 2×10^{11} vg/eye with a prophylactic topical corticosteroid regimen.

[0301] **Cohort 4:** Nine subjects who are diagnosed with wAMD are enrolled into Cohort 4. If signs of choroidal neovascularization exudation requiring rescue therapy are observed in a majority of subjects in Cohorts 2 and 3, the subjects in Cohort 4 are administered a single IVT injection of AAV2.7m8-aflibercept at Dose 3 of 6×10^{10} vg/eye with a topical corticosteroid regimen (Cohort 4b). If signs of choroidal neovascularization exudation requiring rescue therapy are not observed in a majority of subjects in Cohorts 2 and 3, the subjects in Cohort 4 are administered a single IVT injection of AAV2.7m8-aflibercept at Dose 1 of 6×10^{11} vg/eye with a topical corticosteroid regimen (Cohort 4a).

B. Study Visits

[0302] The study visits are as described in Section IV of Example 1 and FIG. 1B.

C. Corticosteroid Regimen

[0303] Subjects in Cohort 2 are administered a prophylactic 13-day oral corticosteroid regimen as described in detail for Cohort in Example 1:

[0304] **Cohort 2:** Subjects in Cohort 2 are administered a single IVT injection of AAV2.7m8-aflibercept at Dose 2 of 2×10^{11} vg/eye with a prophylactic 13-day oral corticosteroid regimen, starting with 60 mg of prednisone 3 days before and 3 days after AAV2.7m8-aflibercept treatment for a total of 6 days. This is followed by a 7-day prednisone taper. A summary of the oral prednisone regimen for Cohort 2 is provided in Table 2 of Example 1.

[0305] Subjects in Cohorts 3 and 4 are administered a prophylactic tapering regimen of topical corticosteroid (difluprednate 0.05% drops) as described below:

[0306] **Cohort 3:** Subjects in Cohort 3 are administered a single IVT injection of AAV2.7m8-aflibercept at Dose 2 of 2×10^{11} vg/eye with a prophylactic topical 4-week tapering corticosteroid regimen.

[0307] **Cohort 4:** Subjects in Cohort 4b are administered a single IVT injection of AAV2.7m8-aflibercept at Dose 3 of 6×10^{10} vg/eye with a prophylactic topical 4-week tapering corticosteroid regimen that can be extended at the discretion of the treating physician. Subjects in Cohort 4a are administered a single IVT injection of AAV2.7m8-aflibercept at Dose 3 of 6×10^{11} vg/eye with a prophylactic topical 4-week tapering difluprednate regimen that can be extended at the discretion of the treating physician.

[0308] A summary of the topical difluprednate regimen is provided in Table 9, below.

Table 9: Summary of topical difluprednate regimen.

Study Weeks	Difluprednate administrations per day
Week 1	4 times
Week 2	3 times
Week 3	2 times
Week 4	1 time
The difluprednate regimen can be extended at the discretion of the treating physician.	

[0309] Initiating immunosuppression immediately after exposure to AAV2.7m8-aflibercept IVT injection is designed to limit the immune response upon exposure to capsid antigens. Subjects self-administer one drop of difluprednate 0.05% solution (2.5 µg difluprednate) to the conjunctival sac of the treated eye 4 times per day for the first week beginning on the day of injection of AAV2.7m8-aflibercept (Day 1, post-injection). This is followed by 3 times per day for second week, 2 times per day for the third week, and 1 time per day for the fourth week. The tapering corticosteroid regimen may be extended at the discretion of the treating physician.

D. Prohibited Medications and Treatments

[0310] The prohibited medications and treatments for this study are as described in Section IV of Example 1.

E. Summary of Study Design for Cohorts 2-4

[0311] A summary of the study design for Cohorts 2-4 is provided in Table 10.

Table 10: Summary of study design for Cohorts 2-4.

Cohort	AAV2.7m8-aflibercept Dose	Corticosteroid Regimen
Cohort 2	2×10^{11} vg/eye	Prophylactic oral prednisone regimen.
Cohort 3	2×10^{11} vg/eye	Prophylactic topical corticosteroid.
Cohort 4a	6×10^{11} vg/eye	Prophylactic topical corticosteroid.
Cohort 4b	6×10^{10} vg/eye	Prophylactic topical corticosteroid.

F. Study Duration

[0312] The duration of subject participation in this study is as described in Section IV of Example 1.

V. Study Assessments

[0313] Assessments performed in this study are as described in Section V of Example 1. In addition, samples are taken of aqueous and vitreous humors.

Aqueous Humor Sampling

[0314] Aqueous humor samples are obtained at screening (prior to aflibercept injection) and on Day 1 (prior to AAV2.7m8-aflibercept injection), Weeks 12, 24, 36, 52, 76, 88, 104, and at any visit requiring the first aflibercept (Eylea) rescue treatment, or early termination. Aqueous humor samples are analyzed for aflibercept, VEGF-A, and NAb concentrations.

Vitreous Humor Sampling

[0315] Vitreous humor samples are obtained at any point during the study when a vitrectomy is performed. Vitreous humor samples are analyzed primarily for aflibercept concentrations. Remaining samples are analyzed for VEGF-A concentrations.

VI. Results***A. Subject Characteristics***

[0316] Disease characteristics and treatment histories for all subjects are recorded. In particular, the date or year of neovascular AMD diagnosis, the number of prior anti-VEGF injections the study eye, the calculated anti-VEGF injection interval average pre-study, the number of anti-VEGF injections received

in the 4 months prior to screening, the date of Screening aflibercept IVT dose, and the date of AAV2.7m8-aflibercept injection are recorded.

B. Safety

[0317] Subjects are closely monitored on the day of the IVT AAV2.7m8-aflibercept administration and thereafter post-treatment.

[0318] The safety of AAV2.7m8-aflibercept is assessed through the collection of vital signs, physical and eye examinations, ECG, pregnancy testing, and laboratory evaluations.

[0319] The incidence, severity, and relationship to treatment of AEs, SAEs, and DLTs are assessed in all subjects.

[0320] The occurrence, severity, and relationship to treatment of intraocular inflammation is assessed in all subjects.

[0321] Visual acuity is assessed in all subjects through BCVA expressed as an ETDRS score (number of letters correctly read) (Vitale *et al.*, (2016) JAMA Ophthalmol 134(9):1041-1047).

[0322] The requirement for anti-VEGF rescue injections is monitored in all subjects.

[0323] The existence of a correlation between preexisting anti-AAV NAb-levels in subject's sera and AEs, SAEs, and DLTs is determined.

[0324] The effect of steroid prophylaxis (topical or oral) on the occurrence and resolution of AEs, SAEs, DLTs, and intraocular inflammation is assessed.

[0325] Standard of care aflibercept IVT injections are used as a rescue treatment.

C. Efficacy Assessments

[0326] Disease stabilization is assessed in all subjects. Retinal thickness, central subfield thickness, fluid and other anatomical features are measured (*e.g.*, by OCT imaging) prior to and after administration of study treatment.

[0327] The efficacy of AAV2.7m8-aflibercept in the treatment of wAMD is assessed by the following measures. A key assessment time point is at 24 weeks. The baseline values of BCVA and SD-OCT refer to the values taken during the screening visit on Days -15 to -7 (*e.g.*, Days -14 to -7) prior to aflibercept injection. The baseline values are used to compare for analysis.

[0328] Vision is assessed primarily through BCVA expressed as an ETDRS score (number of letters correctly read) (Vitale *et al.*, (2016) JAMA Ophthalmol 134(9):1041-1047). Maintenance of vision is classified if the subject loses fewer than 15 letters in the ETDRS score compared to Baseline. Calculated endpoints include the mean change from Baseline, the percent gaining at least 15 letters compared to Baseline, and the percent losing 15 or more letters compared to Baseline.

[0329] FA is performed to assess CNV lesions using a standard technique to evaluate leakage compared to Baseline.

[0330] SD-OCT is performed using approved equipment and standard techniques to evaluate retinal thickness (*e.g.*, central retinal thickness or central subfield thickness), macular volume, and the presence of fluid (*e.g.*, subretinal fluid and intraretinal fluid) and fluid compared to Baseline values.

[0331] The number of aflibercept injections given post AAV2.7m8-aflibercept treatment per subject, over time, from Week 4 to Week 104 are determined. In addition, the time to the first aflibercept injection post treatment with AAV2.7m8-aflibercept and the proportion of subjects who did not require an aflibercept re-treatment are determined.

[0332] The proportion of subjects without IRF over time from week 4 to week 104 s determined.

[0333] The proportion of subjects without SRF over time from week 4 to week 104 is determined.

[0334] The proportion of subjects without a PED over time from week 4 to week 104 among subjects with a PED at baseline is determined.

[0335] Although the present disclosure has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the present disclosure. The disclosures of all patent and scientific literature cited herein are expressly incorporated in the entirety by reference.

CLAIMS

1. A method for treating an ocular neovascular disease in an individual, the method comprising administering a unit dose of about 6×10^{11} vector genomes (vg) or less of recombinant adeno-associated virus (rAAV) particles to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise:
 - a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and
 - b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO. 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein.
2. The method of claim 1, wherein the retinal fluid in the eye of the individual with ocular neovascular disease is reduced.
3. A method for reducing retinal fluid in an eye of an individual with an ocular neovascular disease, the method comprising administering a unit dose of rAAV particles to one eye of the individual, wherein the individual is a human, and wherein the rAAV particles comprise:
 - a) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35 and flanked by AAV2 inverted terminal repeats (ITRs), and
 - b) an AAV2 capsid protein comprising an amino acid sequence LGETTRP (SEQ ID NO. 14) inserted between positions 587 and 588 of the capsid protein, wherein the amino acid residue numbering corresponds to an AAV2 VP1 capsid protein.
4. The method 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.
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. The method of any one of claims 2-6, wherein the retinal fluid in the one eye is reduced by at least about 60%.

8. The method of any one of claims 2-7, wherein the retinal fluid in the one eye is reduced by about 80% compared to the level of retinal fluid in the one eye of the individual prior to administration of the rAAV to the individual.
9. The method of any one of claims 2-8, wherein the retinal fluid is subretinal fluid (SRF) or intraretinal fluid (IRF).
10. The method of any one of claims 2-9, wherein the unit dose of rAAV particles is about 6×10^{11} vector genomes per eye (vg/eye) or less.
11. The method of any one of claims 1-10, wherein the unit dose of rAAV particles is about 6×10^{10} to about 2×10^{11} vector genomes per eye (vg/eye).
12. The method of any one of claims 1-11, wherein the unit dose of rAAV particles is about 2×10^{11} or about 6×10^{10} vector genomes per eye (vg/eye).
13. The method of any one of claims 1-12, further comprising administering a unit dose of rAAV particles to the contralateral eye of the individual.
14. The method of claim 13, wherein 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.
15. The method of claim 14, wherein the unit dose of rAAV particles administered to the contralateral eye of the individual comprises the same or less vector genomes per eye (vg/eye) than the unit dose of rAAV particles administered to the one eye of the individual.
16. The method of claim 13, wherein the administering the unit dose of rAAV particles to the contralateral eye is at least about 2 weeks after administering the unit dose of rAAV particles to the one eye.
17. The method of claim 16, wherein the unit dose of rAAV particles administered to the contralateral eye of the individual comprises more vector genomes per eye (vg/eye) than the unit dose of rAAV particles administered to the one eye of the individual.
18. The method of any one of claims 1-17, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 35.

19. The method of any one of claims 1-18, wherein the polypeptide is aflibercept.
20. The method of any one of claims 1-19, 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.
21. The method of any one of claims 1-20, wherein the nucleic acid comprises, in the 5' to 3' order:
- (a) a first enhancer region;
 - (b) a promoter region;
 - (c) a nucleic acid encoding a polypeptide comprising an amino acid sequence with at least about 95% identity to the amino acid sequence of SEQ ID NO: 35;
 - (d) a 5'UTR region;
 - (e) a second enhancer region; and
 - (f) a polyadenylation site;
- and flanked by AAV2 inverted terminal repeats (ITRs).
22. The method of claim 20 or claim 21, wherein the first enhancer region comprises a CMV sequence comprising the sequence of SEQ ID NO: 22 or a sequence having at least 85% identity thereto.
23. The method of any one of claims 20-22, wherein the promoter region comprises a CMV sequence comprising the sequence of SEQ ID NO: 23 or a sequence having at least 85% identity thereto.
24. The method of any one of claims 20-23, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 35 or a sequence having at least 85% identity thereto.
25. The method of any one of claims 20-24, wherein the polypeptide is aflibercept.
26. The method of any one of claims 20-25, wherein the 5'UTR region comprises, in 5' to 3' order, a TPL sequence comprising the sequence of SEQ ID NO: 24 or a sequence having at least 85% identity thereto, and an eMLP sequence comprising the sequence of SEQ ID NO: 25 or a sequence having at least 85% identity thereto.

27. The method of any one of claims 20-26, wherein the second enhancer region comprises a full EES sequence comprising the sequence of SEQ ID NO: 26 or a sequence having at least 85% identity thereto.
28. The method of any one of claims 20-27, wherein the polyadenylation site comprises a HGH polyadenylation site comprising the sequence of SEQ ID NO: 27 or a sequence having at least 85% identity thereto.
29. The method of any one of claims 1-19, wherein the nucleic acid further comprises (a) a first enhancer region comprising a CMV sequence comprising the sequence of SEQ ID NO: 22 or a sequence having at least 85% identity thereto; (b) a promoter region, comprising a CMV sequence comprising the sequence of SEQ ID NO: 23 or a sequence having at least 85% identity thereto; (c) a 5'UTR region comprising, in 5' to 3' order, a TPL sequence comprising the sequence of SEQ ID NO: 24 or a sequence having at least 85% identity thereto, and an eMLP sequence comprising the sequence of SEQ ID NO: 25 or a sequence having at least 85% identity thereto; (d) a second enhancer region comprising a full EES sequence comprising the sequence of SEQ ID NO: 26 or a sequence having at least 85% identity thereto; and (e) a HGH polyadenylation site comprising the sequence of SEQ ID NO: 27 or a sequence having at least 85% identity thereto.
30. The method of any one of claims 1-29, 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.
31. The method of any one of claims 1-30, wherein the AAV2 capsid protein comprises the amino acid sequence LGETTRP (SEQ ID NO: 14) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13.
32. The method of any one of claims 1-31, wherein the AAV2 capsid protein comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of the AAV2 VP1 comprising the sequence of SEQ ID NO: 13.
33. The method of any one of claims 1-32, wherein the administration of the unit dose of rAAV particles to the one eye and/or the contralateral eye is by intravitreal administration.
34. The method of any one of claims 1-33, wherein the unit dose of rAAV particles is in a pharmaceutical formulation.

35. The method of claim 34, wherein the pharmaceutical formulation comprises the rAAV particles, sodium chloride, sodium phosphate and a surfactant.
36. The method of claim 35, wherein the pharmaceutical formulation comprises about 150 to about 200 mM sodium chloride, about 1 to about 10 mM monobasic sodium phosphate, about 1 to about 10 mM dibasic sodium phosphate, about 0.0005% (w/v) to about 0.005% (w/v) poloxamer 188, and about 6×10^{13} to about 6×10^{10} vector genomes (vg) per mL (vg/mL) of the rAAV particles, wherein the pharmaceutical formulation has a pH of about 7.0 to about 7.5.
37. The method of claim 36, wherein the pharmaceutical formulation comprises about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 6×10^{12} vg/mL of the rAAV particles, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3.
38. The method of claim 36, wherein the pharmaceutical formulation comprises about 180 mM sodium chloride, about 5 mM monobasic sodium phosphate, about 5 mM dibasic sodium phosphate, about 6×10^{11} vg/mL of the rAAV particles, and about 0.001% (w/v) poloxamer 188, wherein the pharmaceutical formulation has a pH of about 7.3.
39. The method of any one of claims 1-38, wherein the unit dose of rAAV particles comprises a volume of about 25 μ L to about 250 μ L.
40. The method of claim 39, wherein the unit dose of rAAV particles comprises a volume of about 100 μ L.
41. The method of claim 39, wherein the unit dose of rAAV particles comprises a volume of about 30 μ L.
42. The method of any one of claims 1-41, wherein the individual received prior treatment for the ocular neovascular disease with an anti-VEGF agent.
43. The method of claim 42, wherein the anti-VEGF agent is aflibercept.
44. The method of any one of claims 1-43, wherein the ocular neovascular disease is wet age-related macular degeneration (AMD), retinal neovascularization, choroidal neovascularization diabetic retinopathy, proliferative diabetic retinopathy, retinal vein occlusion, central retinal vein

occlusion, branched retinal vein occlusion, diabetic macular edema, diabetic retinal ischemia, ischemic retinopathy, diabetic retinal edema, or any combination thereof.

45. The method of any one of claims 1-44, wherein the unit dose of rAAV particles is administered in combination with steroid treatment.

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

47. The method of claim 45 or claim 46, wherein the steroid treatment is a systemic steroid treatment.

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

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

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

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

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

53. The method of any one of claims 50-52, wherein 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.

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

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

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

57. The method of any one of claims 1-56, 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 of retinal thickness compared to the retinal thickness prior to administration of the unit dose of rAAV particles.

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

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

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

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

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

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

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

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

66. The method of anyone of claims 64-65, wherein visual acuity is best corrected visual acuity (BCVA).

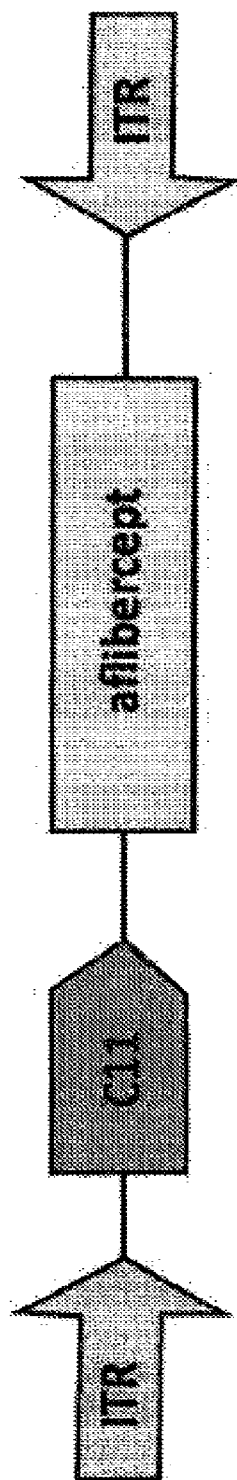


FIG. 1A

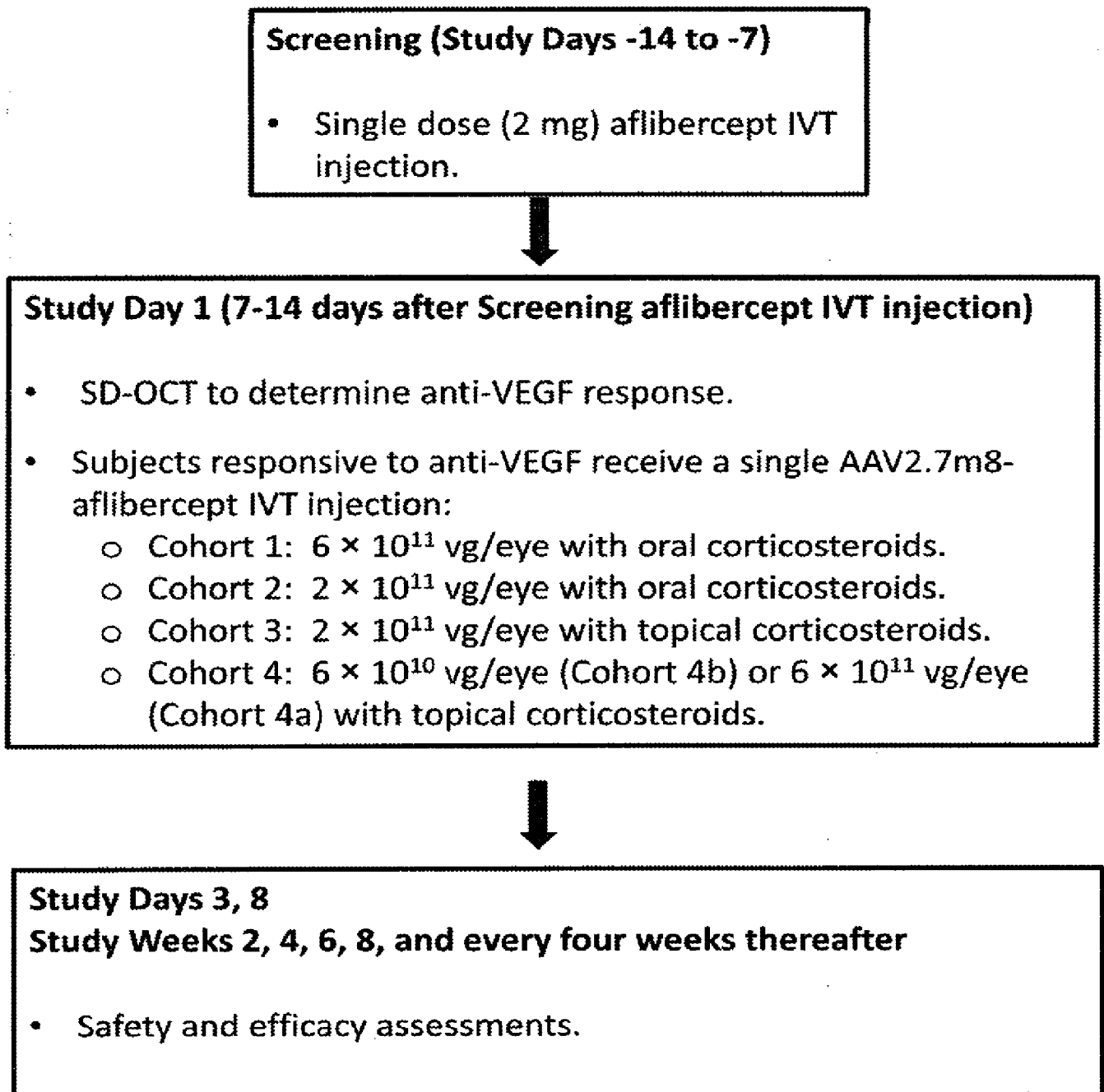
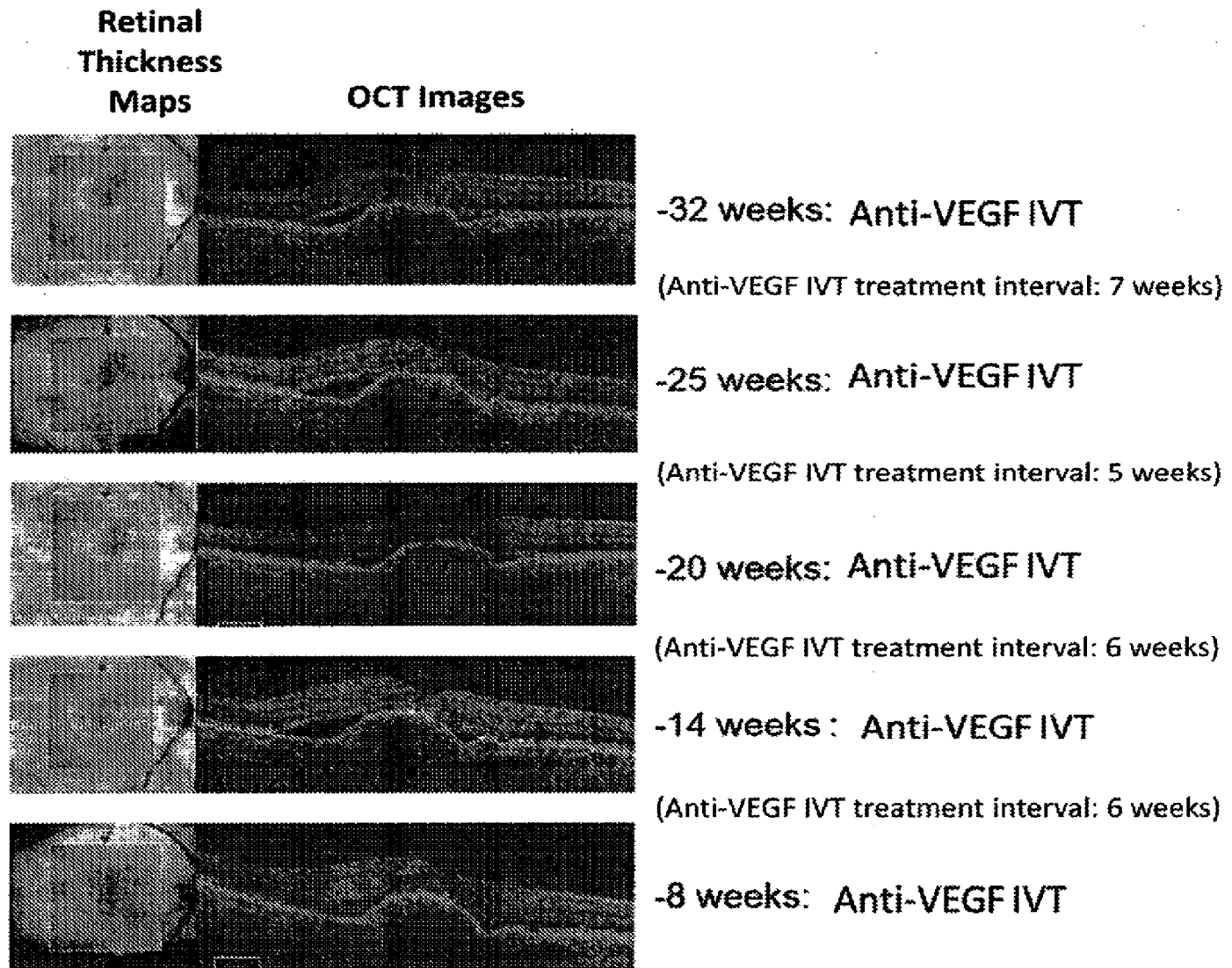
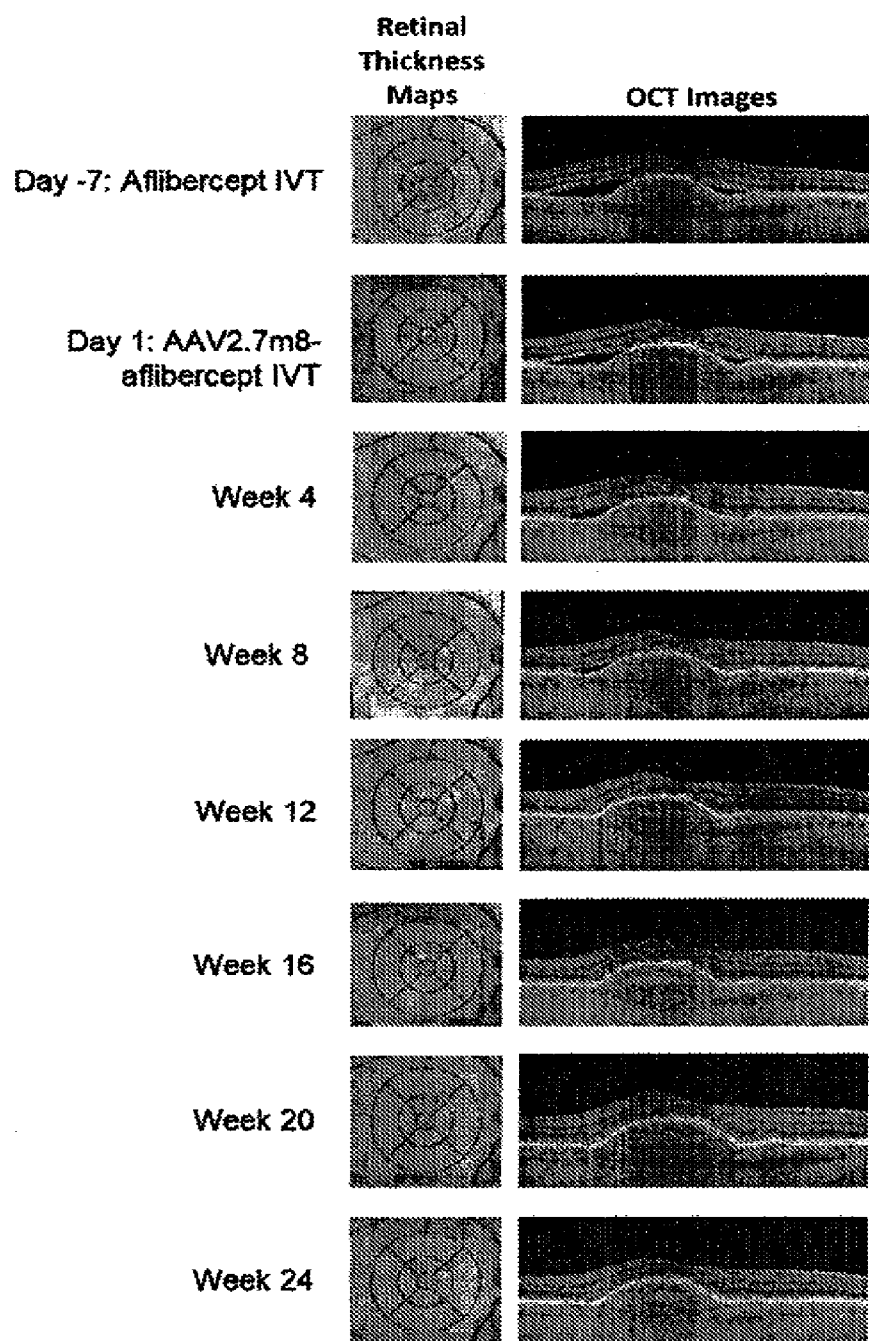
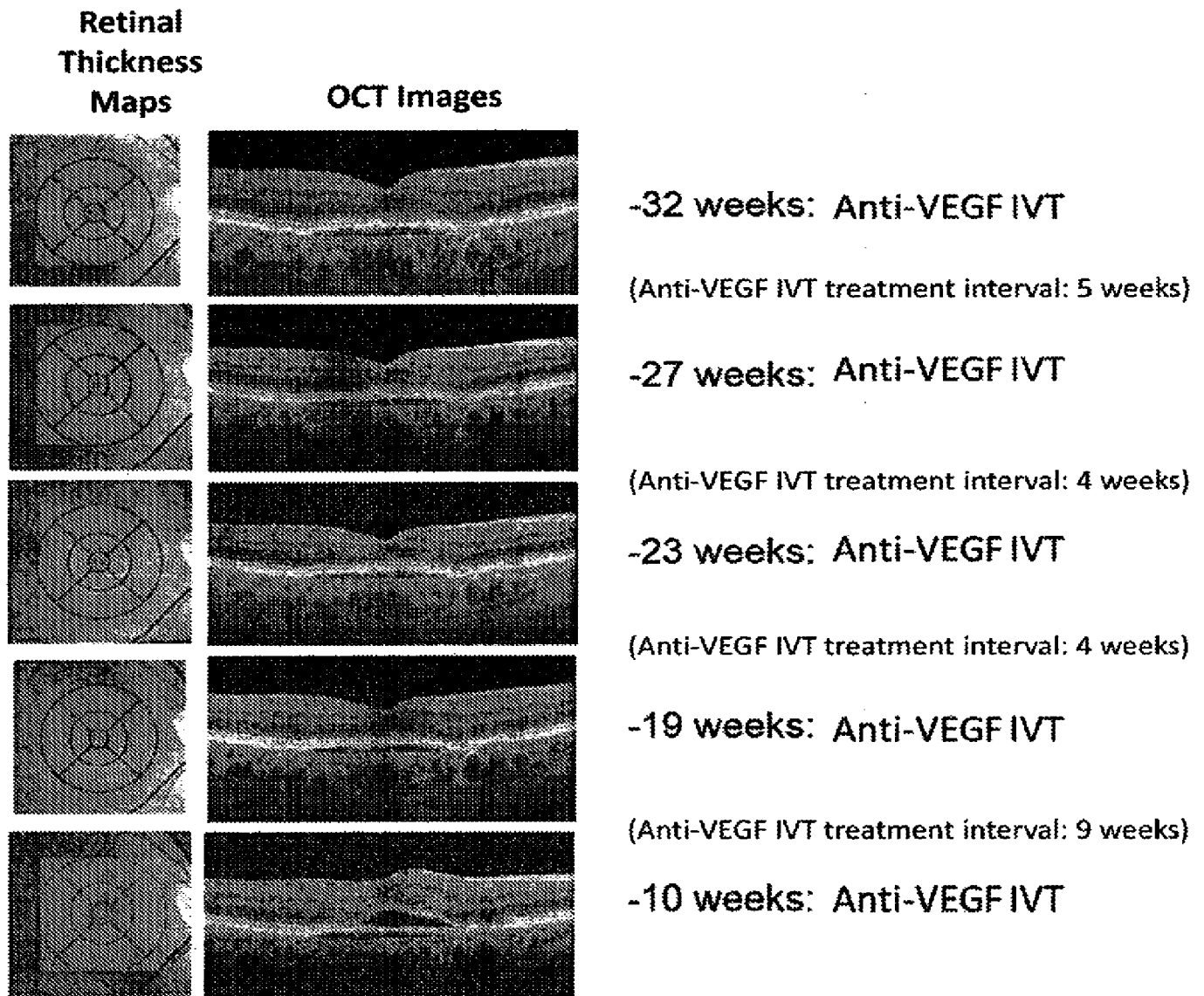


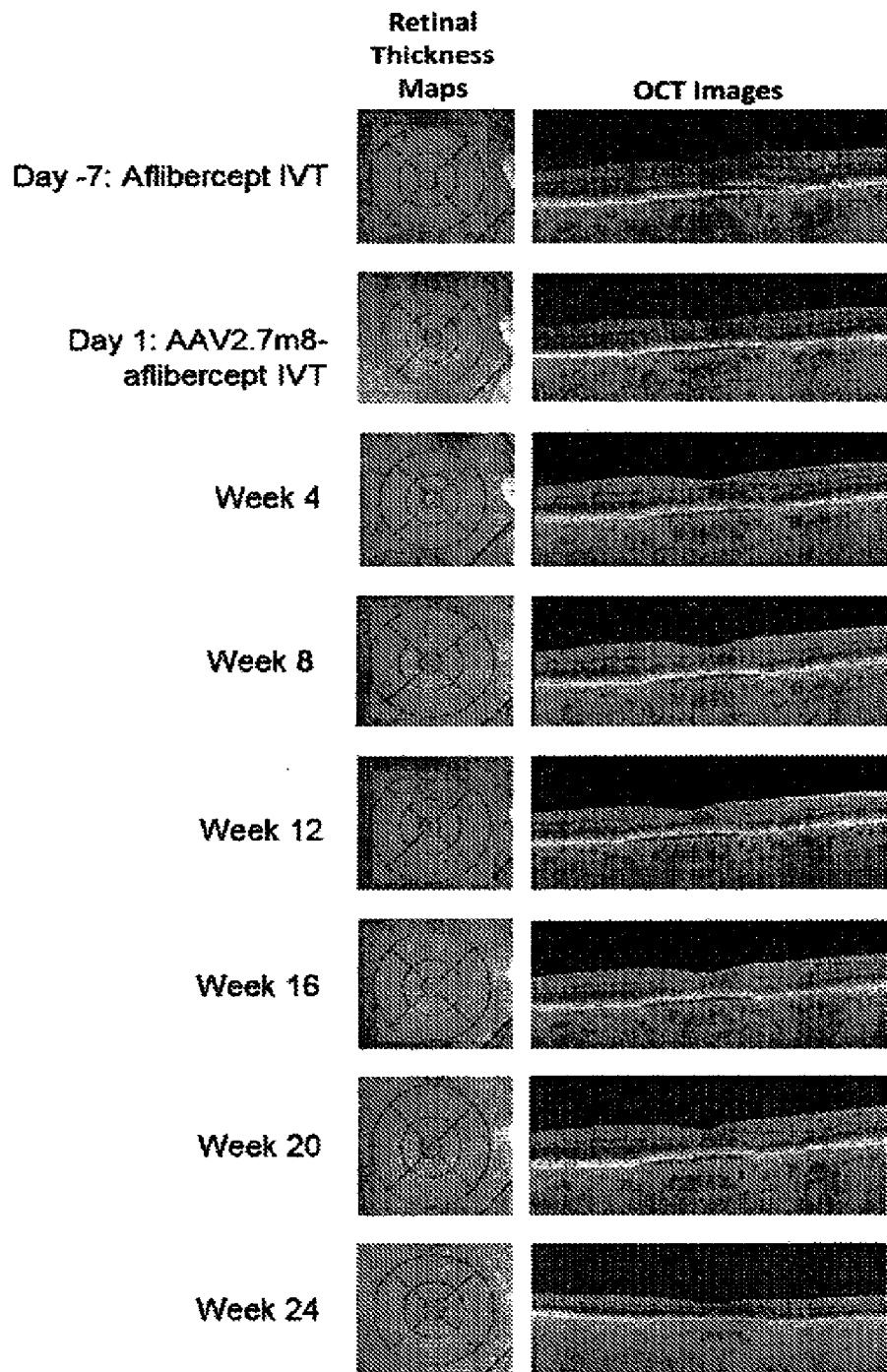
FIG. 1B

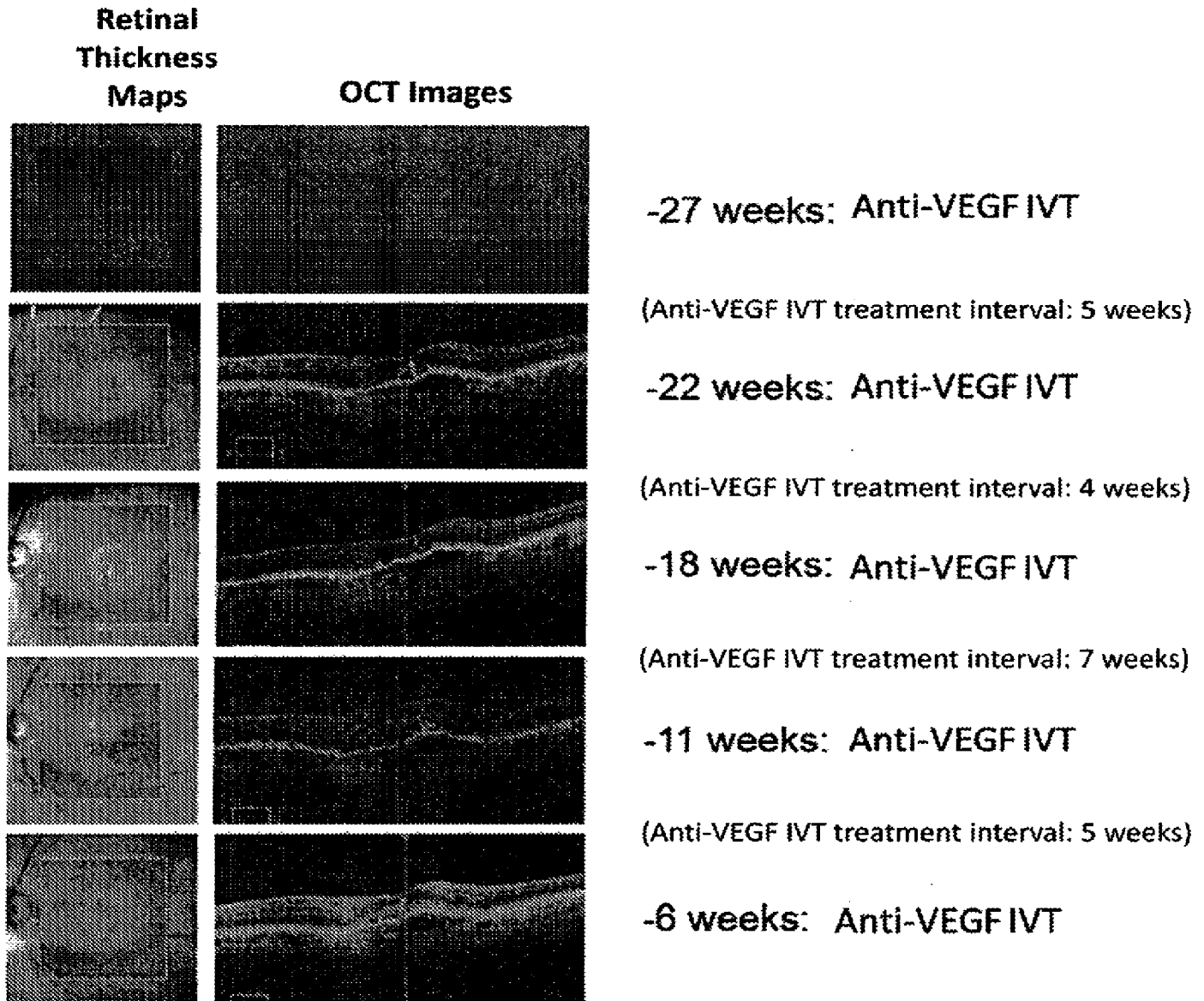
Subject 1 Prior nAMD Treatment Experience**FIG. 2A**

Subject 1 OCT Images by Visit**FIG. 2B**

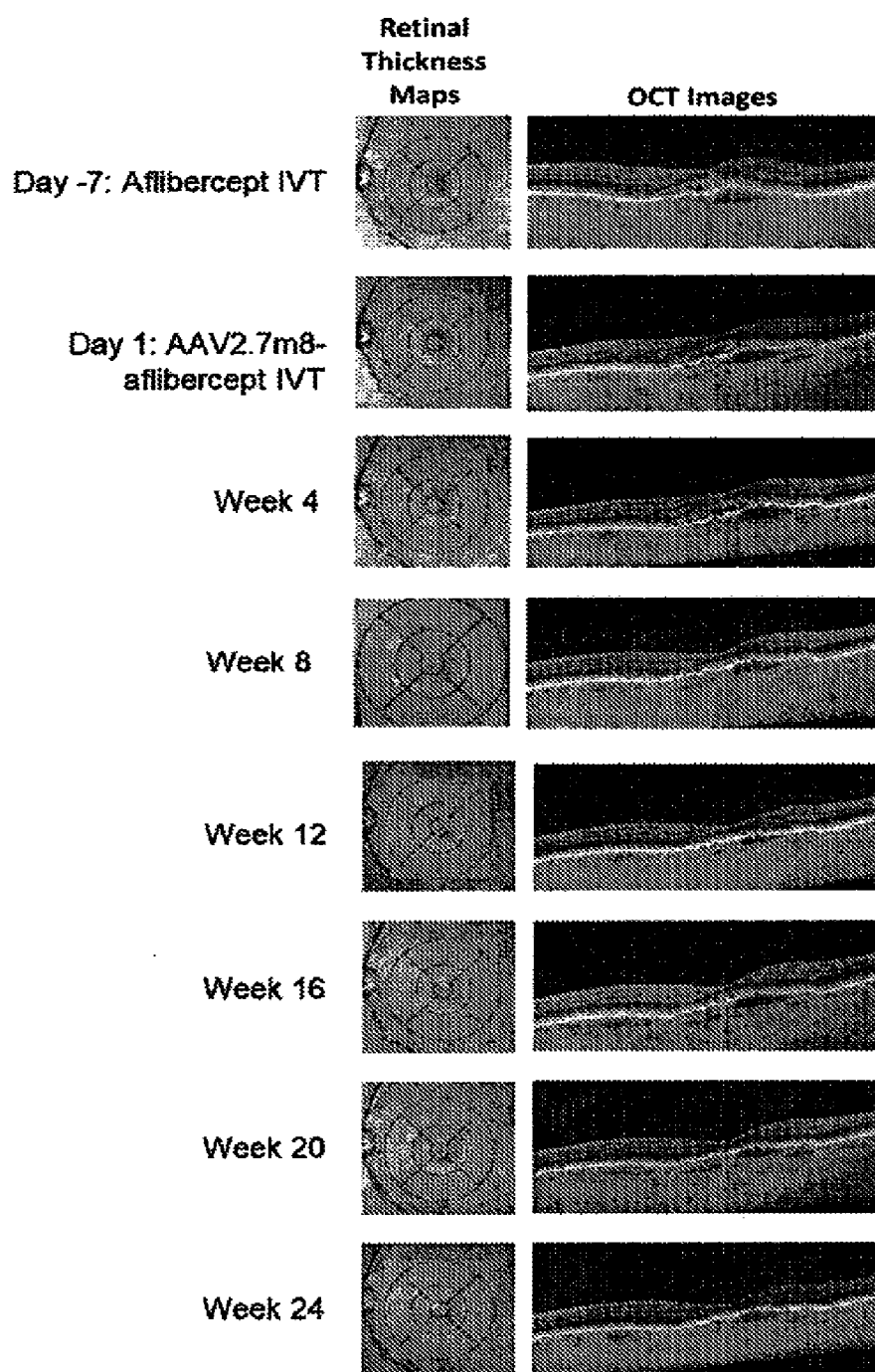
Subject 2 Prior nAMD Treatment Experience**FIG. 2C**

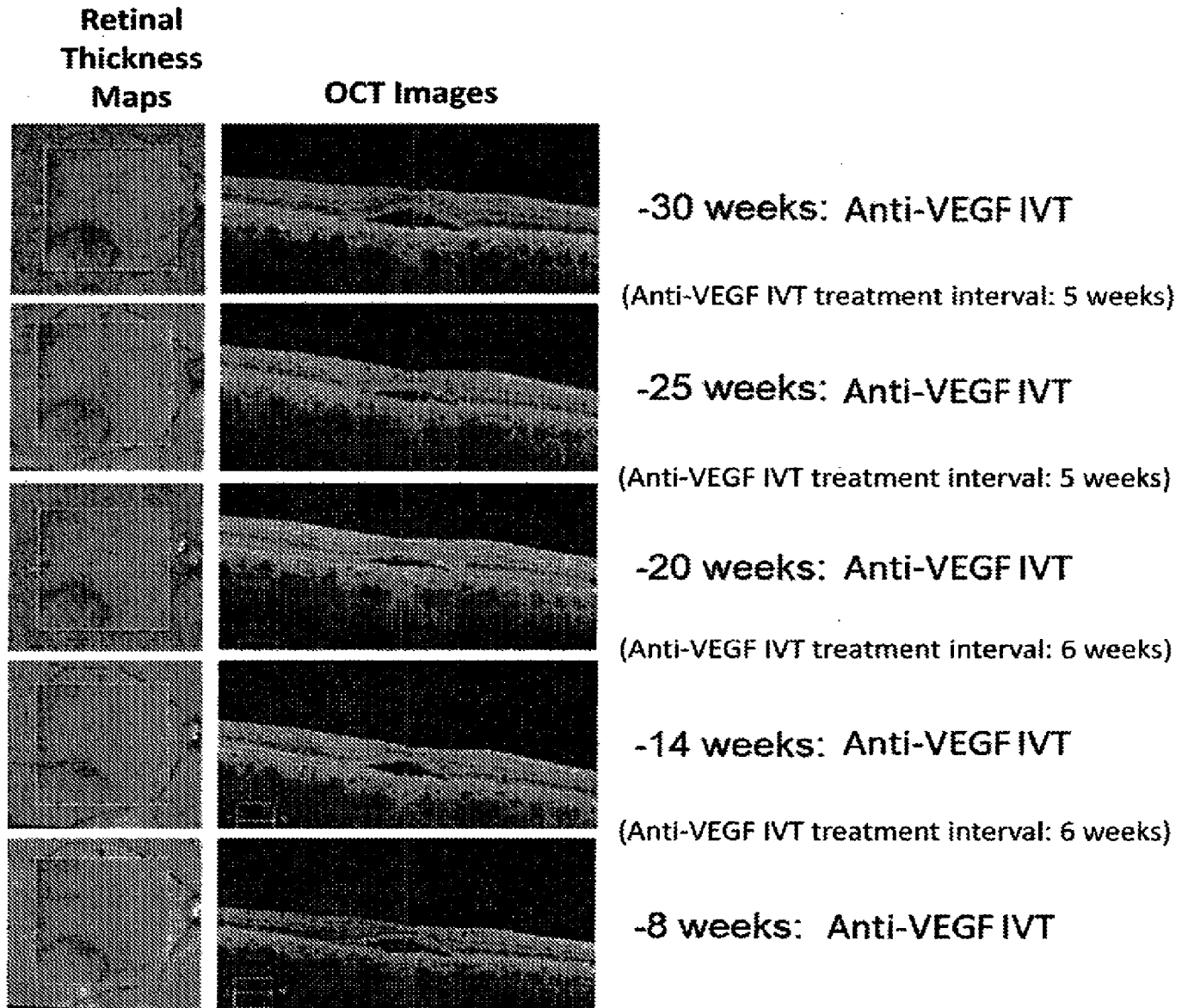
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Subject 2 OCT Images by Visit**FIG. 2D**

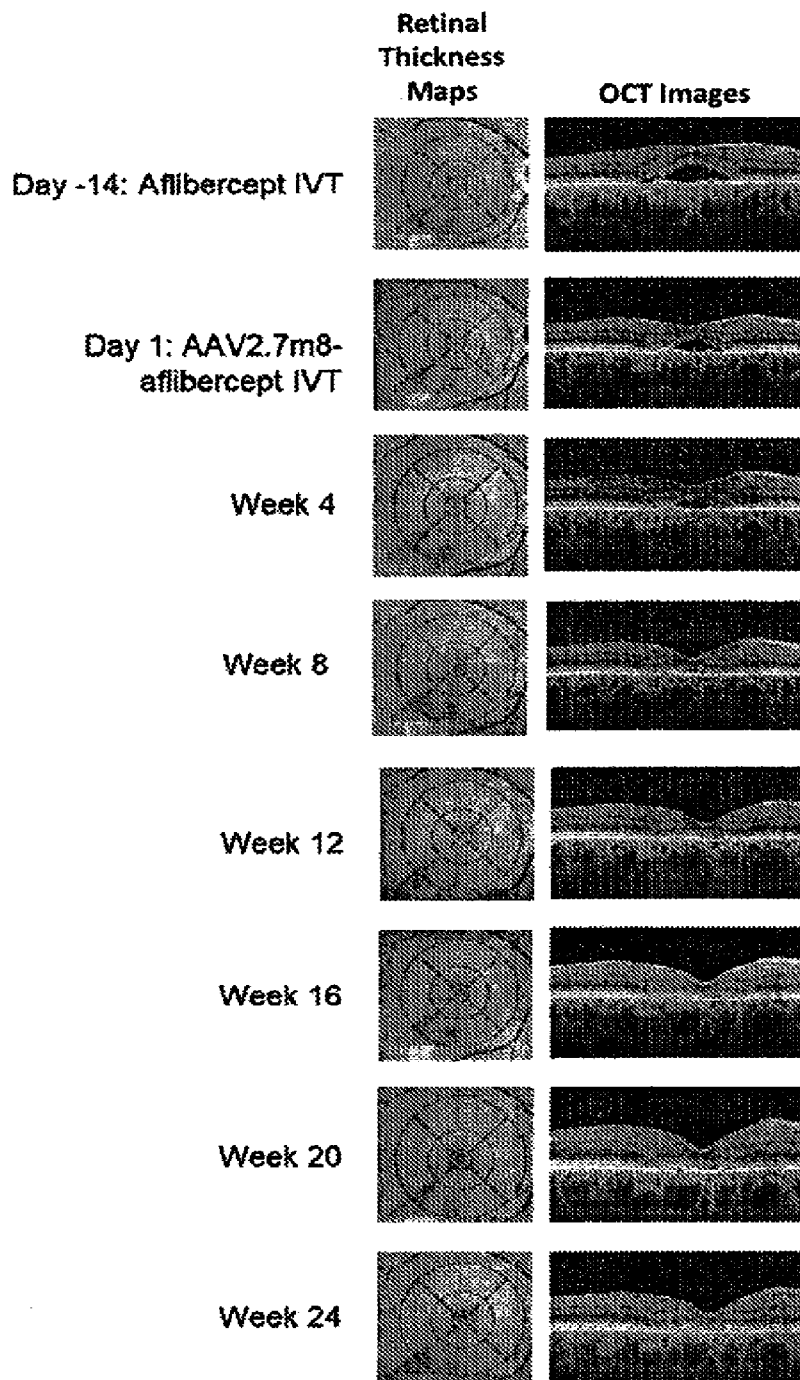
Subject 3 Prior nAMD Treatment Experience**FIG. 2E**

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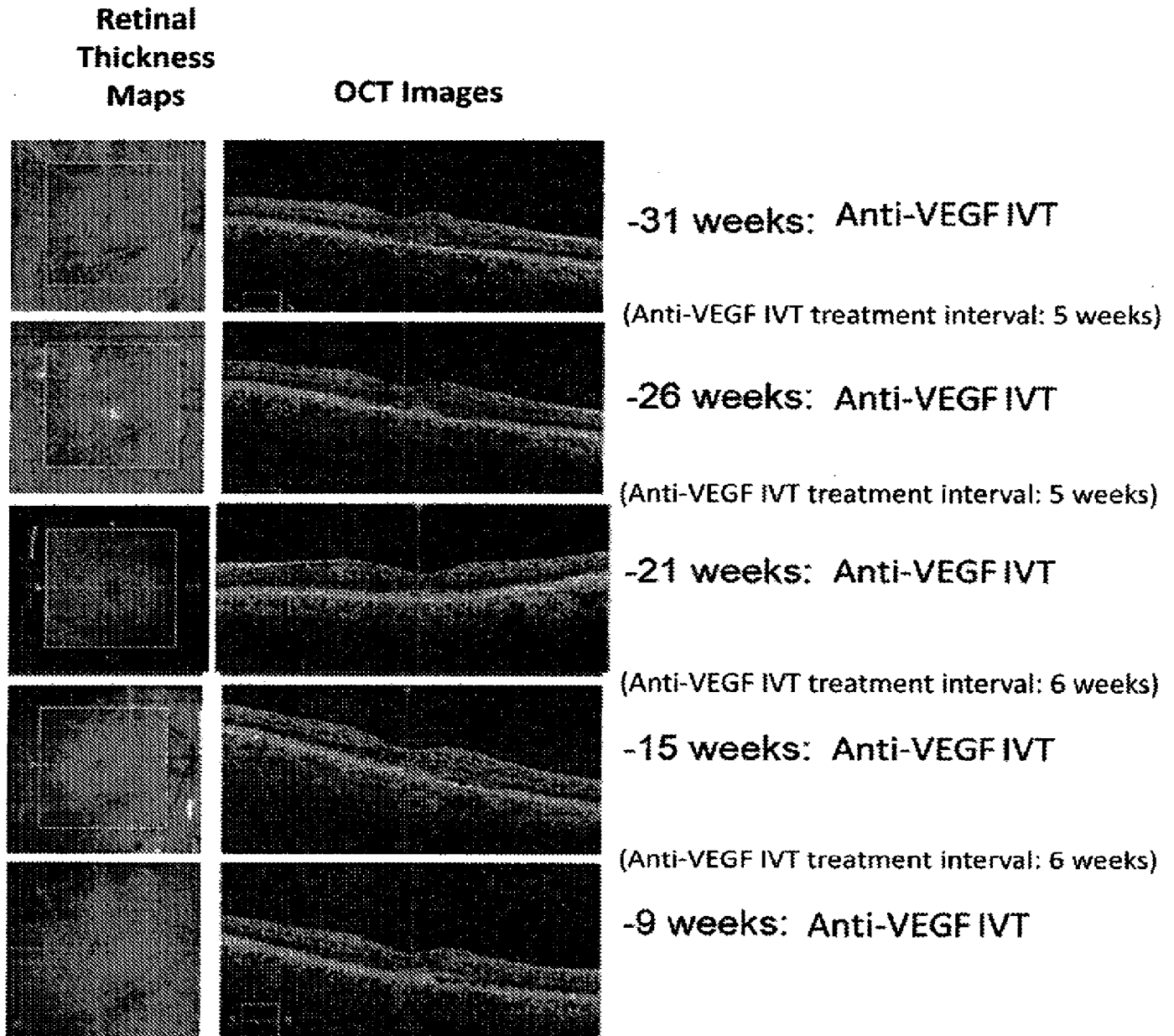
Subject 3 OCT Images by Visit**FIG. 2F**

Subject 4 Prior nAMD Treatment Experience**FIG. 2G**

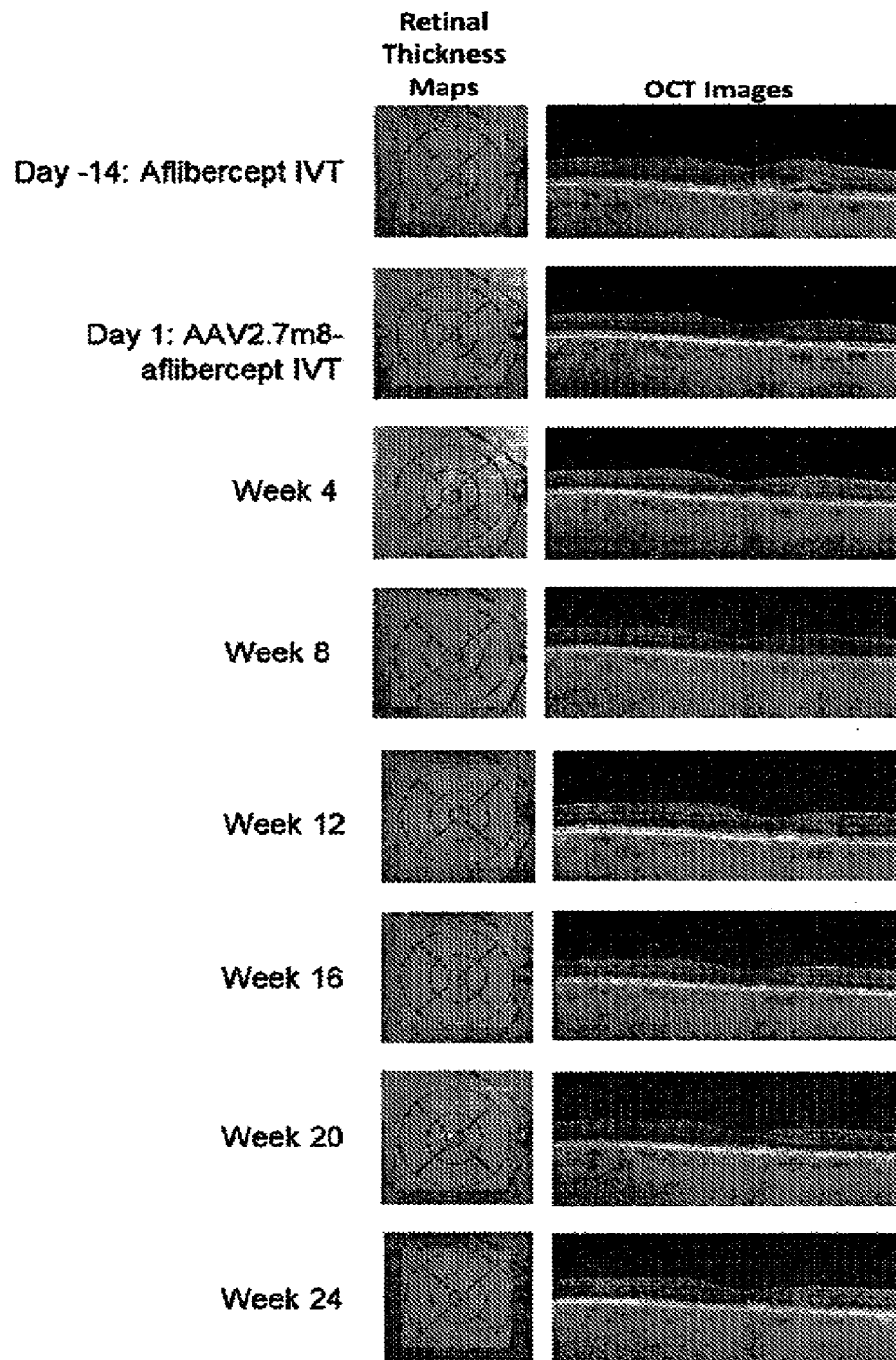
10/20

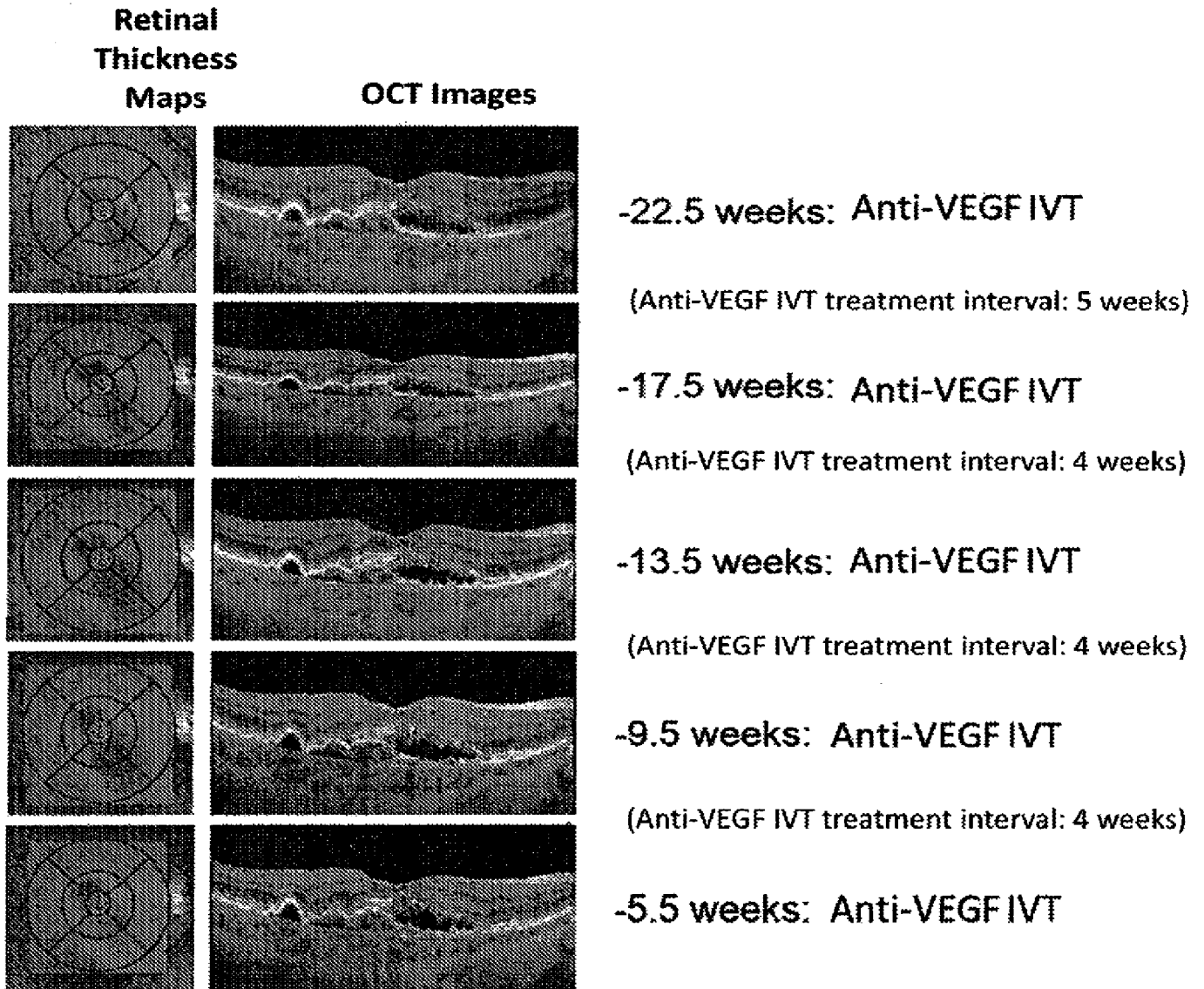
Subject 4 OCT Images by Visit**FIG. 2H**

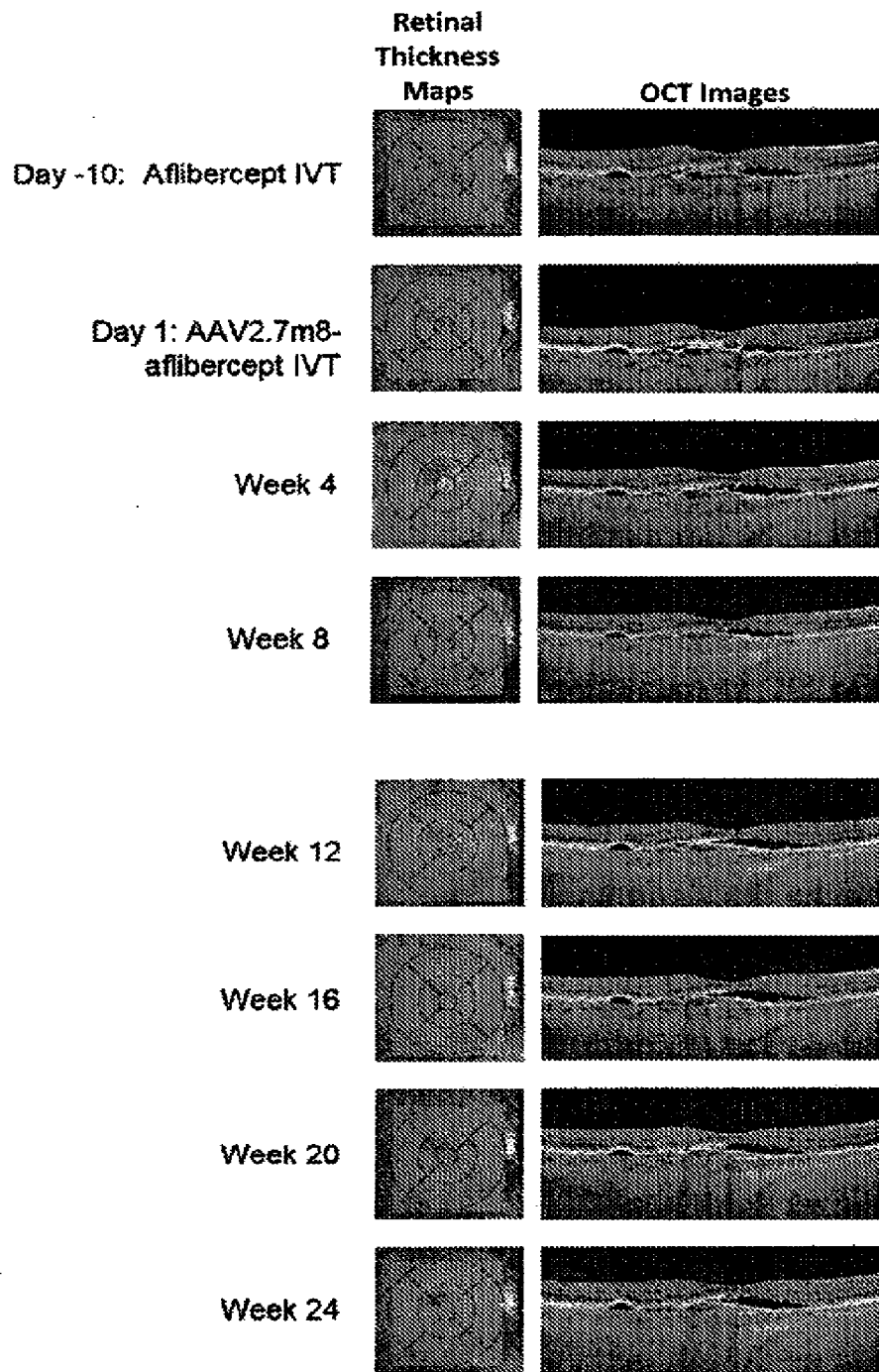
11/20

Subject 5 Prior nAMD Treatment Experience**FIG. 2I**

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Subject 5 OCT Images by Visit**FIG. 2J**

Subject 6 Prior nAMD Treatment Experience**FIG. 2K**

Subject 6 OCT Images by Visit**FIG. 2L**

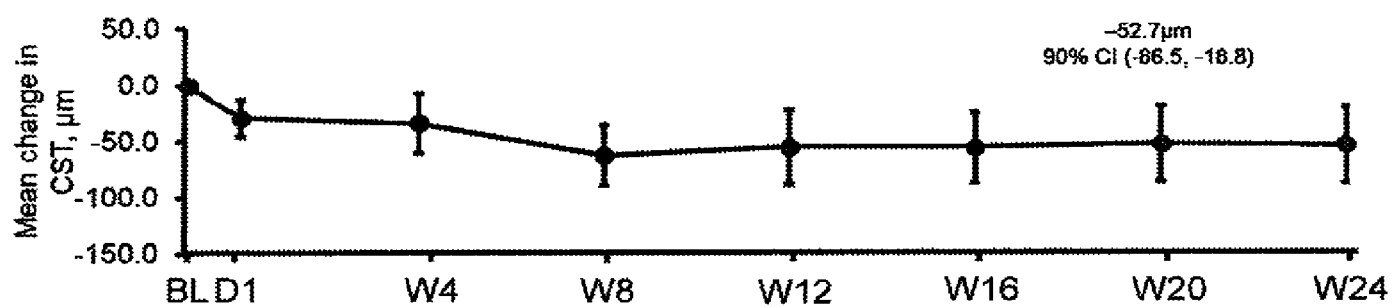


FIG. 3

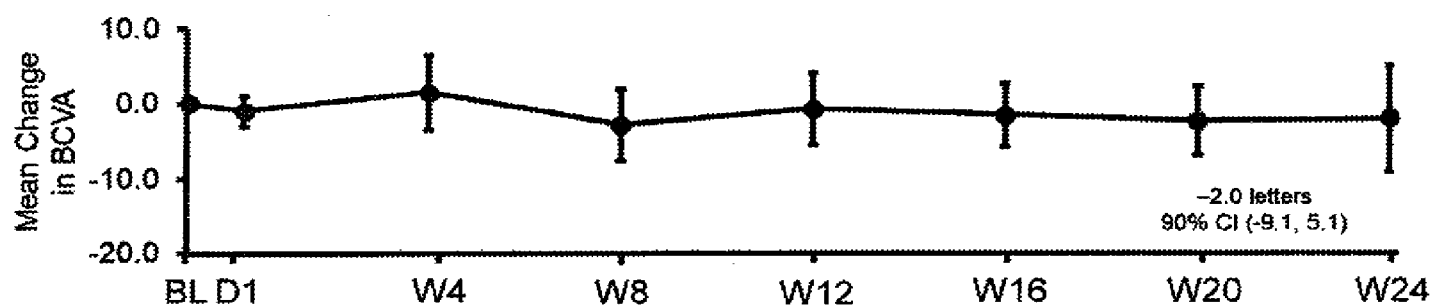


FIG. 4

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ACTTTGATCCCTGATGGAAACGCATAATCTGGGACAGTAGAAAGGGCTTCATC
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CATGCCACCCGTGCCAGCACCTGAACCTCTGGGGGACCGTCAGTCTTCTCTT
CCCCCAAAACCCAGGACACCCCTCATGTATCTCCCGGACCCCTGAGGTCACATG
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FIG. 5

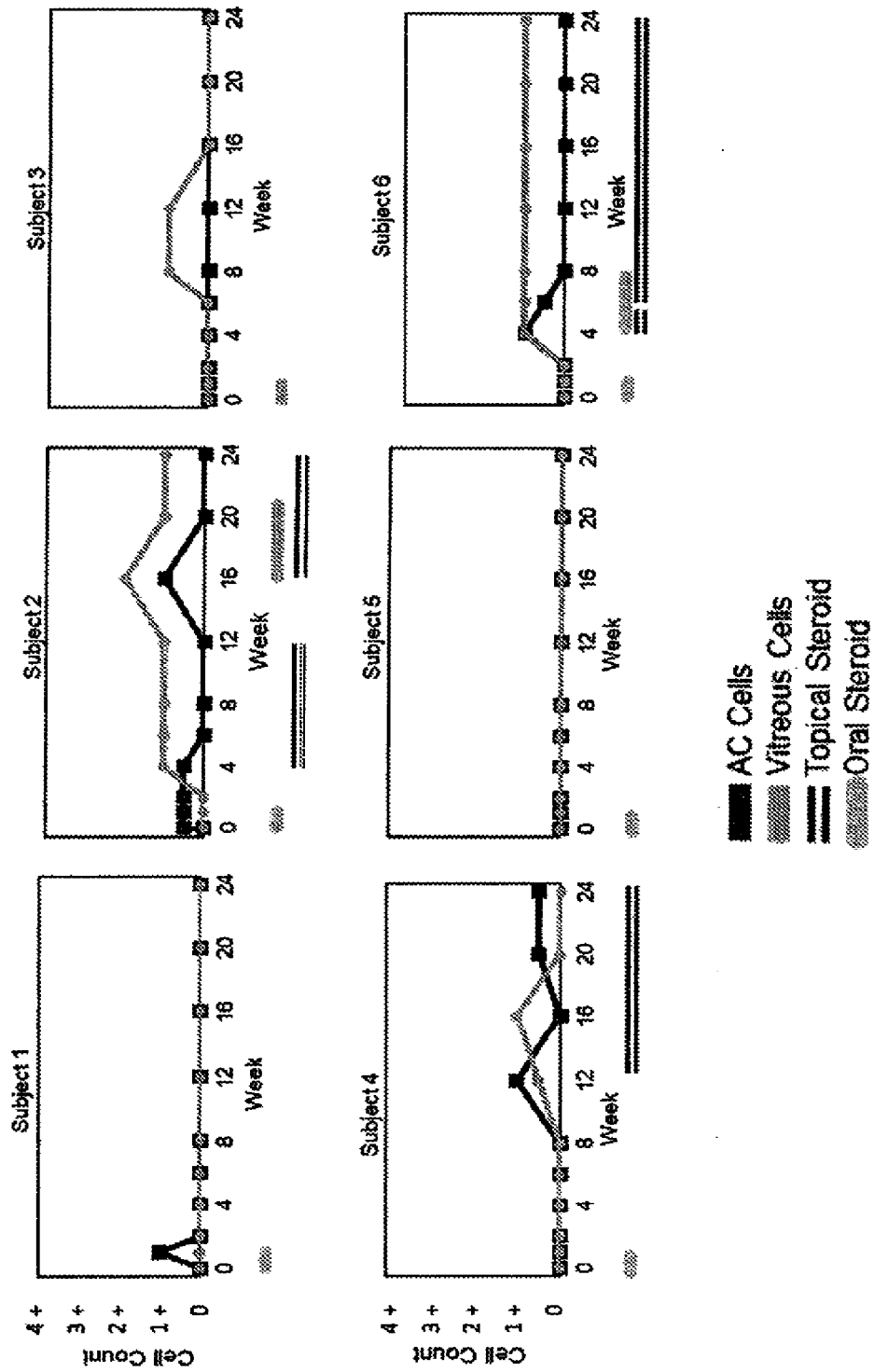


FIG. 6

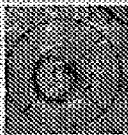
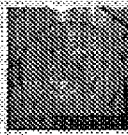
	Subject 1: 44 Weeks Post-AAV2/Ind-omere-p		Subject 2: 40 Weeks Post-AAV2/Ind-omere-p		Subject 3: 36 Weeks Post-AAV2/Ind-omere-p	
Baseline OCT						
Latest OCT						
BCVA Change from Baseline, ETDRS letters	+3		-9		-6	
IVT in 8 months prior to this study, n	5		6		6	
Rescue IVT during this study, n	0		0		0	

FIG. 7A

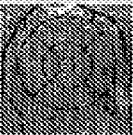
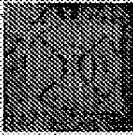
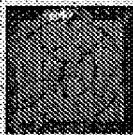
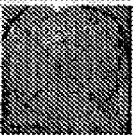
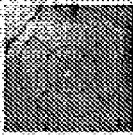
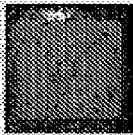
	Subject 4, 32 Weeks Post-AAV2, TMS-diffusion		Subject 5, 28 Weeks Post-AAV2, TMS-diffusion		Subject 6, 28 Weeks Post-AAV2, TMS-diffusion	
Baseline OCT						
Latest OCT						
BCVA Change from Baseline, ETDRS letters	+5		+1		-3	
IVT in 8 months prior to this study, n	6		6		8	
Rescue IVT in this study, n	0		0		0	

FIG. 7B