

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 2.5 μ g.

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).