

CLAIM

What is claimed is:

1. A recombinant adeno-associated virus (rAAV), comprising:
 - (i) an AAV capsid protein, wherein the capsid protein is an AAV2 capsid protein,
an AAV2/3 hybrid capsid protein, an AAV8 capsid protein or a variant thereof; and
 - (ii) an isolated nucleic acid comprising a transgene encoding an anti-vascular
endothelial growth factor (anti-VEGF) agent, the transgene being flanked by adeno-
associated virus (AAV) inverted terminal repeats (ITRs).
2. The rAAV of claim 1, wherein the anti-VEGF agent is a human VEGF decoy receptor.
3. The rAAV of claim 2, wherein the human VEGF decoy receptor comprises extracellular
domain 2 of human VEGF receptor 1.
4. The rAAV of claim 2, wherein the human VEGF decoy receptor comprises extracellular
domains 3 and 4 of human VEGF receptor 2.
5. The rAAV of any one of claims 2-4, wherein the VEGF decoy receptor is capable of
binding to anti-vascular endothelial growth factor (VEGF) and/or placenta growth factor (PlGF).
6. The rAAV of claim 1 or 2, wherein the anti-VEGF agent is a human VEGF receptor
fusion protein.
7. The rAAV of claim 6, wherein the human VEGF receptor fusion protein comprises
extracellular domain 2 of human VEGF receptor 1 fused to extracellular domains 3 and 4 of
human VEGF receptor 2.
8. The rAAV of claim 6, wherein the human VEGF receptor fusion protein comprises
extracellular domain 2 of human VEGF receptor 1 fused to an Fc portion of an immunoglobulin.

9. The rAAV of claim 6, wherein the human VEGF receptor fusion protein comprises extracellular domains 3 and 4 of human VEGF receptor 2 fused to an Fc portion of an immunoglobulin.

10. The rAAV of claim 6, wherein the human VEGF receptor fusion protein comprises extracellular domain 2 of human VEGF receptor 1 fused to extracellular domains 3 and 4 of human VEGF receptor 2, and further fused to an Fc portion of an immunoglobulin.

11. The rAAV of claim 10, wherein the anti-VEGF agent is KH902.

12. The rAAV of claims 10 or 11, wherein the anti-VEGF agent comprises an amino acid sequence at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99% or 100% identical to amino acid sequence of SEQ ID NO: 5, or a portion thereof.

13. The rAAV of claims 10-12, wherein the transgene comprises a nucleic acid at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99% or 100% identical to nucleic acid sequence of SEQ ID NO: 1 or a codon optimized variant thereof.

14. The rAAV of any one of claims 6-13, wherein the anti-VEGF agent is capable of binding to anti-vascular endothelial growth factor (VEGF) and/or placenta growth factor (PlGF).

15. The rAAV of any one of claims 1-14, wherein the isolated nucleic acid further comprises a promoter operably linked to the transgene.

16. The rAAV of claim 15, wherein the promoter comprises a cytomegalovirus (CMV) early enhancer.

17. The rAAV of claim 16, wherein the promoter comprises a chimeric cytomegalovirus (CMV)/Chicken β -actin (CB) promoter.

18. The rAAV of any one of claims 1-17, wherein the transgene comprises one or more introns.

19. The rAAV of claim 18, wherein at least one intron is positioned between the promoter and the nucleic acid sequence encoding the anti-vascular endothelial growth factor (anti-VEGF) agent.

20. The rAAV of any one of claims 1-19, wherein the transgene comprises a Kozak sequence.

21. The rAAV of claim 20, wherein the Kozak sequence is positioned between the intron and the transgene encoding the anti-vascular endothelial growth factor (anti-VEGF) agent.

22. The rAAV of any one of claims 1-21, wherein the transgene comprises a 3' untranslated region (3'UTR).

23. The rAAV of any one of claims 1-22, wherein the transgene further comprises one or more miRNA binding sites.

24. The rAAV of claim 23, wherein the one or more miRNA binding sites are positioned in a 3'UTR of the transgene.

25. The rAAV of claim 23 or 24, wherein the at least one miRNA binding site is an immune cell-associated miRNA binding site.

26. The rAAV of claim 25, wherein the immune cell-associated miRNA is selected from: miR-15a, miR-16-1, miR-17, miR-18a, miR-19a, miR-19b-1, miR-20a, miR-21, miR-29a/b/c, miR-30b, miR-31, miR-34a, miR-92a-1, miR-106a, miR-125a/b, miR-142-3p, miR-146a, miR-150, miR-155, miR-181a, miR-223 and miR-424, miR-221, miR-222, let-7i, miR-148, and miR-152.

27. The rAAV of any one of claims 1-26, wherein the ITRs are adeno-associated virus ITRs of a serotype selected from the group consisting of AAV1 ITR, AAV2 ITR, AAV3 ITR, AAV4 ITR, AAV5 ITR, and AAV6 ITR.

28. The rAAV of any one of claims 1-27, wherein the isolated nucleic acid comprises a nucleic acid sequence at least 80%, 90%, 99% or 100% identical to the nucleic acid sequence of SEQ ID NO: 2.

29. The rAAV of any one of claims 1-28, wherein the capsid protein comprises an amino acid sequence at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99% or 100% identical to amino acid sequences of v224 capsid protein, v326 capsid protein, v358 capsid protein, v46 capsid protein, v56 capsid protein, v66 capsid protein, v67 capsid protein, v81 capsid protein, v439 capsid protein, v453 capsid protein, v513 capsid protein, v551 capsid protein, v556 capsid protein, v562 capsid protein, or v598 capsid protein.

30. The rAAV of claim 29, wherein the capsid protein comprises an amino acid sequence at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99% or 100% identical to amino acid sequences of v224 capsid protein, v326 capsid protein, or v56 capsid protein.

31. The rAAV of any one of claims 1-30, wherein the capsid protein has tropism for ocular tissue.

32. The rAAV of claim 35, wherein the ocular tissue comprises ocular neurons, retina, sclera, choroid, retina, vitreous body, macula, fovea, optic disc, lens, pupil, iris, aqueous fluid, cornea, conjunctiva ciliary body, or optic nerve.

33. The rAAV of any one of claims 1-32, wherein the rAAV is a single-stranded AAV (ssAAV) or a self-complementary AAV (scAAV).

34. The rAAV of any one of claims 1-33, wherein the capsid protein variants is capable of increasing rAAV packaging efficiency as compared to the wild-type capsid protein they derive from.

35. The rAAV of claim 34, wherein the AAV2 capsid protein variant is capable of increasing rAAV packaging efficiency by at least 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold or more as compared to the wild-type AAV2 capsid protein.

36. The rAAV of claim 34, wherein the AAV2/3 hybrid capsid protein variant is capable of increasing rAAV packaging efficiency by at least 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold or more as compared to the wild-type AAV3b capsid protein.

37. A recombinant adeno-associated virus comprising:

(i) a rAAV capsid protein, wherein the capsid protein is a variant of AAV8 capsid protein, AAV2 capsid protein and/or an AAV2/3 hybrid capsid protein or a variant thereof; and
(ii) a recombinant adeno-associated virus (rAAV) vector comprising a nucleic acid comprising, in 5' to 3' order:

(a) a 5' AAV ITR;

(b) a CMV enhancer;

(c) a CBA promoter;

(d) a chicken beta-actin intron;

(d) a Kozak sequence;

(e) a transgene encoding an anti-VEGF agent, wherein the anti-VEGF agent is encoded by the nucleic acid sequence in SEQ ID NO: 1;

(f) a rabbit beta-globin polyA signal tail; and

(g) a 3' AAV ITR.

38. A host cell comprising the rAAV of any one of claims 1 to 37.

39. The host cell of claim 38, wherein the host cell is a mammalian cell, yeast cell, bacterial cell, or insect cell.

40. A pharmaceutical composition comprising the rAAV of any one of claims 1-37.

41. The pharmaceutical composition of claim 40, further comprises a pharmaceutically acceptable carrier.

42. The pharmaceutical composition of claims 40 or 41, wherein the pharmaceutical composition is formulated for intravitreal injection, intravenous injection, intratumoral injection, intrastromal injection, or intramuscular injection.

43. A method of inhibiting VEGF or PlGF activity in a subject in need thereof, the method comprising

administering to the subject an effective amount of the rAAV of any one of claims 1-37,
5 or the pharmaceutical composition of any one of claims 40-42.

44. A method of delivering an anti-VEGF agent in a subject in need thereof, the method comprising

administering to the subject an effective amount of the rAAV of any one of claims 1-37,
10 or the pharmaceutical composition of any one of claims 40-42.

45. A method of treating a neovascularization associated disease, an angiogenesis associated disease or a VEGF associated disease in a subject in need thereof, the method comprising

administering to the subject an effective amount of the rAAV of any one of claims 1-37,
15 or the pharmaceutical composition of any one of claims 40-42.

46. The rAAV of any one of claims 1-37, or the pharmaceutical composition of any one of claims 40-42 for use in inhibiting VEGF activity in a subject in need thereof.

47. The rAAV of any one of claims 1-37, or the pharmaceutical composition of any one of claims 40-42 for use in delivering an anti-VEGF agent in a subject in need thereof.

48. The rAAV of any one of claims 1-37, or the pharmaceutical composition of any one of claims 40-42 for use in treating a neovascularization associated disease, an angiogenesis
25 associated disease, or a VEGF-associated disease in a subject in need thereof.

49. The method or the use of claims 43-48, wherein the delivery of the anti-VEGF agent result in 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 100% inhibition of VEGF activity.

50. The method or the use of any one of claims 43-49, wherein the subject is a non-human mammal.

51. The method or the use of claim 50, wherein the non-human mammal is mouse, rat, cat, dog, sheep, rabbit, horse, cow, goat, pig, guinea pig, hamster, chicken, turkey, or a non-human primate.

5 52. The method or the use of any one of claims 43-49, wherein the subject is a human.

53. The method or the use of claim 52, wherein the subject is diagnosed or is suspect of having an angiogenesis associated disease or a VEGF associated disease.

10 54. The method or the use of claim 53, wherein the VEGF associated disease is tumor, cancer, retinopathy, wet age-related macular degeneration (wAMD), macular edema, choroidal neovascularization, or corneal neovascularization.

15 55. The method or the use of any one of claims 43-54, wherein the administration is systemic administration, optionally wherein the administration is intravenous injection.

56. The method or the use of any one of claims 43-55, wherein the administration is direct administration to ocular tissue, optionally wherein the direct administration is intravitreal injection, intraocular injection, intrastromal injection, or topical administration.53.

20 57. The method or the use of any one of claims 43-56, wherein the administration results in delivery of the transgene to ocular tissue.

25 58. The method or the use of claim 57, the ocular tissue comprises ocular neurons, retina, sclera, choroid, retina, vitreous body, macula, fovea, optic disc, lens, pupil, iris, aqueous fluid, cornea, conjunctiva ciliary body, or optic nerve.

30 59. The method or the use of any one of claims 43-58, wherein the administration results in inhibition of VEGF in the subject for at least 5 days, 10 days, 15 day, 20 days, 1 month, two months, or longer post administration.

60. A method of treating a corneal neovascularization (CoNV) in a subject in need thereof, the method comprising administering to the subject an effective amount of the rAAV of any one of claims 1-37, or the pharmaceutical composition of any one of claims 40-42.

5 61. The method of claim 60, wherein the rAAV comprises an AAV8 capsid protein.

62. The method of claims 60 or 61, wherein the administration results in delivery of the anti-VEGF agent in corneal cells.

10 63. The method of claim 62, wherein the administration results in delivery of the anti-VEGF agent in keratocytes of the cornea.

64. The method of any one of claims 60-63, wherein the rAAV is administered once.

15 65. The method of any one of claims 60-64, wherein the administration results in expression of the anti-VEGF agent in corneal cells for longer than three months, six months, a year, or longer.

20 66. The method claim 35, wherein the administration results in inhibition of VEGF in the subject for 1 month, two months, three months, six months, a year or longer post administration.

67. The method of any one of claims 60-66, wherein the administration is intrastromal injection.

25 68. The method of any one of claims 60-67, wherein the subject is human.

69. The method of any one of claims 60-68, wherein the corneal neovascularization is acute corneal neovascularization or chronic corneal neovascularization.

CLAIM

What is claimed is:

1. A recombinant adeno-associated virus (rAAV), comprising:
 - (i) an AAV capsid protein, wherein the capsid protein is an AAV8 capsid protein or a variant thereof, an AAV2 capsid protein, an AAV2/3 hybrid capsid protein,; and
 - (ii) an isolated nucleic acid comprising a transgene encoding an anti-vascular endothelial growth factor (anti-VEGF) agent, the transgene being flanked by adeno-associated virus (AAV) inverted terminal repeats (ITRs).

2. The rAAV of claim 1, wherein the anti-VEGF agent is a human VEGF decoy receptor or a human VEGF receptor fusion protein.

3. The rAAV of claim 2, wherein the human VEGF receptor fusion protein comprises extracellular domain 2 of human VEGF receptor 1 fused to extracellular domains 3 and 4 of human VEGF receptor 2.

4. The rAAV of claim 2, wherein the human VEGF receptor fusion protein comprises extracellular domain 2 of human VEGF receptor 1 fused to extracellular domains 3 and 4 of human VEGF receptor 2, and further fused to an Fc portion of an immunoglobulin.

5. The rAAV of claim 4, wherein the anti-VEGF agent is KH902.

6. The rAAV of claims 4 or 5, wherein the anti-VEGF agent comprises an amino acid sequence at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99% or 100% identical to amino acid sequence of SEQ ID NO: 5, or a portion thereof.

7. The rAAV of any one of claims 4-6, wherein the transgene comprises a nucleic acid at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99% or 100% identical to nucleic acid sequence of SEQ ID NO: 1 or a codon optimized variant thereof.

8. The rAAV of any one of claims 2-7, wherein the anti-VEGF agent is capable of binding to anti-vascular endothelial growth factor (VEGF) and/or placenta growth factor (PlGF).

9. The rAAV of any one of claims 1-8, wherein the isolated nucleic acid further comprises a promoter operably linked to the transgene.

10. The rAAV of claim 9, wherein the promoter comprises a cytomegalovirus (CMV) early enhancer.

11. The rAAV of claim 10, wherein the promoter comprises a chimeric cytomegalovirus (CMV)/Chicken β -actin (CB) promoter.

12. The rAAV of any one of claims 1-11, wherein the transgene comprises one or more introns.

13. The rAAV of claim 12, wherein at least one intron is positioned between the promoter and the nucleic acid sequence encoding the anti-vascular endothelial growth factor (anti-VEGF) agent.

14. The rAAV of any one of claims 1-13, wherein the transgene comprises a Kozak sequence.

15. The rAAV of claim 14, wherein the Kozak sequence is positioned between the intron and the transgene encoding the anti-vascular endothelial growth factor (anti-VEGF) agent.

16. The rAAV of any one of claims 1-15, wherein the ITRs are adeno-associated virus ITRs of a serotype selected from the group consisting of AAV1 ITR, AAV2 ITR, AAV3 ITR, AAV4 ITR, AAV5 ITR, and AAV6 ITR.

17. The rAAV of any one of claims 1-16, wherein the isolated nucleic acid comprises a nucleic acid sequence at least 80%, 90%, 99% or 100% identical to the nucleic acid sequence of SEQ ID NO: 2.
- 5 18. The rAAV of any one of claim 1-17, wherein the nucleic acid sequence is at least 80%, 90%, 99% or 100% identical to the nucleic acid sequence of SEQ ID NO: 30.
19. The rAAV of any one of claims 1-17, wherein the capsid protein comprises an amino acid sequence at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at
10 least 99% or 100% identical to amino acid sequences of v224 capsid protein, v326 capsid protein, v358 capsid protein, v46 capsid protein, v56 capsid protein, v66 capsid protein, v67 capsid protein, v81 capsid protein, v439 capsid protein, v453 capsid protein, v513 capsid protein, v551 capsid protein, v556 capsid protein, v562 capsid protein, or v598 capsid protein.
- 15 20. The rAAV of claim 19, wherein the capsid protein comprises an amino acid sequence at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99% or 100% identical to amino acid sequences of v224 capsid protein, v326 capsid protein, or v56 capsid protein.
- 20 21. The rAAV of any one of claims 1-20, wherein the capsid protein has tropism for ocular tissue.
22. The rAAV of claim 21, wherein the ocular tissue comprises ocular neurons, retina, sclera, choroid, retina, vitreous body, macula, fovea, optic disc, lens, pupil, iris, aqueous fluid, cornea,
25 conjunctiva ciliary body, or optic nerve.
23. The rAAV of any one of claims 1-22, wherein the rAAV is a single-stranded AAV (ssAAV) or a self-complementary AAV (scAAV).
- 30 24. A recombinant adeno-associated virus comprising:

(i) a rAAV capsid protein, wherein the capsid protein is a variant of AAV8 capsid protein, AAV2 capsid protein and/or an AAV2/3 hybrid capsid protein or a variant thereof; and
(ii) a recombinant adeno-associated virus (rAAV) vector comprising a nucleic acid comprising, in 5' to 3' order:

- 5 (a) a 5' AAV ITR;
 (b) a CMV enhancer;
 (c) a CBA promoter;
 (d) a chicken beta-actin intron;
 (d) a Kozak sequence;
10 (e) a transgene encoding an anti-VEGF agent, wherein the anti-VEGF agent is encoded by the nucleic acid sequence in SEQ ID NO: 1;
 (f) a rabbit beta-globin polyA signal tail; and
 (g) a 3' AAV ITR.

15 25. A host cell comprising the rAAV of any one of claims 1 to 24.

26. The host cell of claim 25, wherein the host cell is a mammalian cell, yeast cell, bacterial cell, or insect cell.

20 27. A pharmaceutical composition comprising the rAAV of any one of claims 1-24.

28. The pharmaceutical composition of claim 27, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

25 29. Use of the rAAV of any one of claims 1-24, or the pharmaceutical composition of claim 27 or 28 in the manufacture of a medicament for inhibiting VEGF or PlGF activity in a subject in need thereof, wherein an effective amount of the rAAV or the pharmaceutical composition is to be administered to the subject.

30 30. Use of the rAAV of any one of claims 1-24, or the pharmaceutical composition of claim 27 or 28 in the manufacture of a medicament for delivering an anti-VEGF agent in a subject in

need thereof, wherein an effective amount of the rAAV or the pharmaceutical composition is to be administered to the subject.

31. Use of the rAAV of any one of claims 1-24, or the pharmaceutical composition of claim 27 or 28 in the manufacture of a medicament for treating a neovascularization associated disease, an angiogenesis associated disease or a VEGF associated disease in a subject in need thereof, wherein an effective amount of the rAAV or the pharmaceutical composition is to be administered to the subject.

32. The use of any one of claims 29-31, wherein the delivery of the anti-VEGF agent result in 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 100% inhibition of VEGF activity.

33. The use of any one of claims 29-31, wherein the subject is diagnosed or is suspect of having an angiogenesis associated disease or a VEGF associated disease.

34. The use of claim 33, wherein the VEGF associated disease is tumor, cancer, retinopathy, wet age-related macular degeneration (wAMD), macular edema, choroidal neovascularization, or corneal neovascularization.

35. The rAAV of any one of claims 1-24, or the pharmaceutical composition of claim 27 or 28 for use in therapy.