COMPOSITIONS AND METHODS FOR REDUCING OCULAR NEOVASCULARIZATION

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/351,231, filed June 16, 2016, the disclosure of which is incorporated herein by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[0002] Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. VEGF can be a part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. The normal function of VEGF can be to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels to bypass blocked vessels.

[0003] Overexpression of VEGF can contribute to various disease states and conditions in mammals. Expression of VEGF in certain cancers can allow the cancer cells to grow and metastasize. Overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body.

[0004] VEGF and VEGF receptors (VEGFRs) are implicated in a number of diseases, including the development of choroidal neovascularization (CNV) and age-related macular degeneration. Examples of eye diseases or conditions associated with VEGF and/or VEGFR activity include neovascular (wet) age-related macular degeneration (AMD), macular edema following retinal vein occlusion (RVO), diabetic macular edema (DME), diabetic retinopathy (DR) in patients with DME, ischemic retinopathy, intraocular neovascularization, dry-AMD, retinal neovascularization, diabetic retina ischemia, diabetic retinal edema, proliferative diabetic retinopathy, central retinal vein occlusion, and branched retinal vein occlusion.

SUMMARY

[0005] While some protein- or antibody-based injection therapies are available for the treatment of AMD, e.g., ranibizumab and bevacizumab, a gene therapy method of delivering an anti-VEGF agent into an eye can provide an improved treatment option for patients because gene therapy can provide prolonged or sustained release of the therapeutic agent in vivo without requiring repeated injections, which can increase the risks of inflammation, infection, and other adverse effects in some patients. Additionally, by not requiring repeated injections, gene therapy addresses the patient compliance and adherence challenge associated with therapies that require repeated injections, as non-compliance can result in vision loss and deterioration of the eye disease or

condition. The rate of non-compliance and non-adherence to treatment regimens that require repeated or frequent trips to medical offices for administration is higher among elderly patients, who are most impacted by AMD. Delivering a therapeutic agent into an eye of a patient via gene therapy can thus provide a safer, potentially more cost-effective, and more convenient treatment option for patients, and improve patient outcomes by addressing the non-compliance and non-adherence problem.

[0006] The present disclosure relates to pharmaceutical compositions and methods of prevention or treatment of ocular neovascularization, such as AMD and CNV, in a subject (e.g., a human subject) by administering subretinally or intravitreally a pharmaceutical composition comprising a pharmaceutically effective amount of a vector or viral particles comprising a nucleic acid encoding an anti-VEGF agent, such as sFlt-1, ranibizumab, or bevacizumab.

[0007] Disclosed is a method of treating an eye disease or condition, the method comprising administering a unit dose of a pharmaceutical suspension to a primate subject by injection to an eye, wherein a unit dose of the pharmaceutical suspension comprises: between 1E12 to 1E13 vector genomes of rAAV having a variant capsid protein comprising an insertion of amino acid sequence selected from LGETTRP, NETITRP, KAGQANN, KDPKTTN, KDTDTTR, RAGGSVG, AVDTTKF, and STGKVPN at a position that corresponds to amino acids 570-611 of capsid protein VP1 in AAV2; and a heterologous sequence encoding an anti-vascular endothelial growth factor (anti-VEGF) polypeptide. In some cases, the unit dose comprises between 2E12 to 6E12 vector genomes. In some cases, the subject is a non-human primate. In some cases, the subject is a human. In some cases, the eye condition or disease is neovascular (wet) age-related macular degeneration (AMD), macular edema following retinal vein occlusion, diabetic macular edema (DME), retinal vein occlusion, or diabetic retinopathy associated with DME. In some cases, the eye condition or disease is choroidal neovascularization or AMD. In some cases, administering the suspension results in a reduction in percentage of grade IV lesions by at least 5% as compared to a vehicle control, as measured by color fundus photography. In some cases, the reduction in percentage of grade IV lesions is at least 10%. In some cases, the unit dose comprises a volume that is not more than 100 μL. In some cases, the unit dose comprises a volume that is not more than 50 μL. In some cases, the insertion is LGETTRP at a position between amino acids 587 and 588 in AAV2. In some cases, the subject is responsive to at least one of ranibizumab, bevacizumab, and sVEGFR-1. In some cases, the subject has been pre-treated with ranibizumab or bevacizumab. In some instances, the injection is intravitreal. In some instances, the injection is subretinal. In some cases, the administering by injection occurs not more than once in at least 2 years. In some cases, the administering by injection occurs not more than once in at least 5 years. In some cases, the administering is a one-

time administration. In some cases, the method further comprises agitating the suspension to ensure even distribution prior to the administering step. In some cases, the method further comprises warming the suspension to room temperature prior to the administering step. In some cases, the suspension further comprises a surfactant. In some cases, the surfactant is selected from polysorbates, sodium dodecyl sulfate, sodium lauryl sulfate, lauryl dimethyl amine oxide, polyethoxylated alcohols, polyoxyethylene sorbitan, octoxynol, Brij, pluronic, and polyoxyl castor oil. In some cases, the suspension further comprises phenol, mannitol, sorbitol, or sodium chloride. In some cases, the method further comprises administering an antibiotic solution or an atropine sulfate ointment after the injection. In some cases, the antibiotic solution comprises ciprofloxacin. In some cases, the anti-VEGF polypeptide is a humanized monoclonal antibody. In some cases, the anti-VEGF polypeptide is an antibody fragment or Fab. In some cases, the humanized monoclonal antibody is ranibizumab or bevacizumab. In some cases, the anti-VEGF polypeptide is a soluble, truncated form of VEGF receptor 1 (sVEGFR-1).

[0007a] In one aspect, there is provided use of a unit dose of a pharmaceutical composition in the manufacture of a medicament for treating an eye disease or condition, wherein the medicament is to be administered by intravitreal injection to an eye of a primate subject in need thereof, and wherein the pharmaceutical composition comprises:
a) a rAAV2 variant comprising an amino acid sequence LGETTRP inserted between positions 587 and 588 of capsid protein VP1, a nucleic acid comprising a sequence having at least 95% homology to SEQ ID NO: 9 and a sequence having at least 95% homology to SEQ ID NO: 10, and
(b) a pharmaceutically acceptable excipient.

[0008] Disclosed is a method of treating an eye condition or disease, the method comprising: agitating a suspension composition, comprising: a rAAV having a variant capsid protein comprising an insertion of amino acid sequence selected from LGETTRP, NETITRP, KAGQANN, KDPKTTN, KDTDTTR, RAGGSVG, AVDTTKF, and STGKVPN at a position that corresponds to amino acids 570-611 of capsid protein VP1 in AAV2; and a heterologous sequence encoding an anti-vascular endothelial growth factor (anti-VEGF) polypeptide; and administering the suspension composition to an eye of a human subject via injection. In some cases, the insertion is LGETTRP between amino acids 587 and 588 of AAV2. In some cases, the subject is characterized as having been pre-treated with ranibizumab or bevacizumab. In some cases, the subject is responsive to at least one of ranibizumab and bevacizumab. In some cases, the anti-VEGF polypeptide is a humanized monoclonal antibody. In some cases, the anti-VEGF polypeptide is an antibody fragment or Fab. In some cases, the humanized monoclonal antibody is ranibizumab or bevacizumab. In some cases, the anti-VEGF polypeptide is a soluble, truncated form of VEGF receptor 1 (sVEGFR-1). In some cases, the volume administered to the subject is not more than 50 μL. In some cases, the volume administered to the subject is not more than 100 μL. In some cases, the volume comprises a unit dose of between 1E12 to 1E13 vector genomes. In some cases, the volume comprises a unit dose of between 2E12 to 6E12 vector genomes. In some case, the administering step occurs not more than once in at least 2 years. In some cases, the administering step is a one-time injection. In some cases, the method further comprises assaying the subject for responsiveness to at least one approved therapy before administering the composition. In some cases, the approved therapy comprises ranibizumab and bevacizumab. In some cases, the suspension comprises a

pharmaceutically acceptable excipient. In some cases, the excipient comprises a surfactant or a stabilizer. In some cases, the surfactant is selected from polysorbates, sodium dodecyl sulfate, sodium lauryl sulfate, lauryl dimethyl amine oxide, polyethoxylated alcohols, polyoxyethylene sorbitan, octoxynol, Brij, pluronic, and polyoxyl castor oil. In some cases, the pharmaceutically acceptable excipient comprises phenol, mannitol, sorbitol, or sodium chloride. In some cases, the eye condition or disease is neovascular (wet) age-related macular degeneration (AMD), macular edema following retinal vein occlusion, diabetic macular edema (DME), retinal vein occlusion, or diabetic retinopathy associated with DME. In some cases, the eye condition or disease is choroidal neovascularization or AMD. In some cases, the injection is intravitreal. In some instances, the injection is subretinal. In some cases, the insertion is LGETTRP at a position between amino acids 587 and 588 in AAV2. In some cases, the method further comprises warming the suspension to room temperature before administering.

[0009] Disclosed is a pharmaceutical composition comprising a unit dose of a suspension, comprising: a rAAV having a variant capsid protein comprising an insertion of amino acid sequence selected from LGETTRP, NETITRP, KAGQANN, KDPKTTN, KDTDTTR, RAGGSVG, AVDTTKF, and STGKVPN at a position that corresponds to amino acids 570-611 of capsid protein VP1 in AAV2; and a heterologous sequence encoding an anti-vascular endothelial growth factor (anti-VEGF) polypeptide. In some cases, the unit dose is between 1E12 to1E13 vector genomes. In some cases, the unit dose is between 2E12 to 6E12 vector genomes. In some cases, the suspension is refrigerated. In some cases, a kit comprises the pharmaceutical composition and a solution for diluting the pharmaceutical composition. In some cases, the solution comprises a buffer, salt, alcohol, a surfactant, or any combination thereof. In some cases, the kit further comprises a syringe. In some cases, the anti-VEGF polypeptide is a humanized monoclonal antibody. In some cases, the anti-VEGF polypeptide is an antibody fragment or Fab. In some cases, the humanized monoclonal antibody is ranibizumab or bevacizumab. In some cases, the anti-VEGF polypeptide is a soluble, truncated form of VEGF receptor 1 (sVEGFR-1). In some cases, the insertion is LGETTRP at a position between amino acids 587 and 588 in AAV2.

INCORPORATION BY REFERENCE

[0010] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

WHAT IS CLAIMED IS:

1. Use of a unit dose of a pharmaceutical composition in the manufacture of a medicament for treating an eye disease or condition, wherein the medicament is to be administered by intravitreal injection to an eye of a primate subject in need thereof, and wherein the pharmaceutical composition comprises:

   a) a rAAV2 variant comprising an amino acid sequence LGETTRP inserted between positions 587 and 588 of capsid protein VP1, a nucleic acid comprising a sequence having at least 95% homology to SEQ ID NO: 9 and a sequence having at least 95% homology to SEQ ID NO: 10, and

   (b) a pharmaceutically acceptable excipient.

2. The use of claim 1, wherein the unit dose is between 2E12 to 6E12 vector genomes.

3. The use of claim 1 or 2, wherein the subject is a human.

4. The use of any one of claims 1-3, wherein the eye condition or disease is neovascular (wet) age-related macular degeneration (AMD), macular edema following retinal vein occlusion, diabetic macular edema (DME), retinal vein occlusion, or diabetic retinopathy associated with DME.

5. The use of any one of claims 1-3, wherein the eye condition or disease is choroidal neovascularization or AMD.

6. The use of any one of claims 1-5, wherein injection of the medicament results in a reduction in percentage of grade IV lesions by at least 5% as compared to a vehicle control, as measured by color fundus photography.

7. The use of claim 6, wherein the reduction in percentage of grade IV lesions is at least 10%.

8. The use of any one of claims 1-7, wherein the unit dose has a volume that is not more than 100 μL.

9. The use of claim 8, wherein the unit dose has a volume that is not more than 50 μL.

10. The use of any one of claims 1-9, wherein the subject is responsive to at least one of ranibizumab, bevacizumab, and sVEGFR-1.

11. The use of any one of claims 1-10, wherein the subject has been pre-treated with ranibizumab or bevacizumab.

12. The use of any one of claims 1-11, wherein the injection occurs not more than once in at least 2 years.

13. The use of claim 12, wherein the injection occurs not more than once in at least 5 years.

14. The use of any one of claims 1-11, wherein the injection is a one-time injection.

15. The use of any one of claims 1-14, further comprising agitating the composition to ensure even distribution prior to the injection.

16. The use of any one of claims 1-15, further comprising warming the composition to room temperature prior to the injection.

17. The use of any one of claims 1-16, wherein the composition further comprises a surfactant.

18. The use of claim 17, wherein the surfactant is selected from polysorbates, sodium dodecyl sulfate, sodium lauryl sulfate, lauryl dimethyl amine oxide, polyethoxylated alcohols, polyoxyethylene sorbitan, octoxynol, Brij, pluronic, and polyoxyl castor oil.

19. The use of any one of claims 1-18, wherein the composition further comprises phenol, mannitol, sorbitol, or sodium chloride.

20. The use of any one of claims 1-18, wherein an antibiotic solution or an atropine sulfate ointment is to be administered after the injection.

21. The use of claim 20, wherein the antibiotic solution comprises ciprofloxacin.

22. The use of any one of claims 1-21, wherein the rAAV2 variant comprises a nucleic acid comprising the sequence of SEQ ID NO: 9 and the sequence of SEQ ID NO: 10.