health system in general and to the extent possible should be reduced. The limitations of currently available therapy for CNV secondary to AMD have created a need in the art for alternative approaches which address the high frequency of treatments required and the invasiveness of the treatment procedure. Neovascularization involving VEGF elevation can also lead to other ocular pathologies, such as diabetic retinopathy, diabetic macular edema (DME), and retinal vein occlusions (RVO). These diseases lead to retinal neovascularization and vision loss. VEGF inhibitors such as Lucentis® have demonstrated efficacy in DME and RVO, and, like with wet AMD, require frequent intravitreal administration in order to maintain benefit.

SUMMARY OF THE DISCLOSURE

[0003a] In an aspect of the present disclosure, there is provided a use of an rAAV comprising a nucleic acid sequence encoding an anti-VEGF protein in the manufacture of a pharmaceutical composition for the treatment of ocular neovascularization in an eye of a human subject suffering from ocular neovascularization, said eye of the human subject having previously received a first intravitreal injection of a VEGF inhibitor in an amount such that the VEGF inhibitor is present in the eye of the subject at a concentration sufficient to prevent progression of the neovascularization at the time of administration of the pharmaceutical composition, wherein a unit dose of the pharmaceutical composition comprises at least 1×10^6 and at most 1×10^{15} vector genomes of the rAAV and a pharmaceutically acceptable carrier and is sufficient to cause elevated levels of said anti-VEGF protein in the eye of said human subject when measured at least one year after administration of the pharmaceutical composition, and wherein the eye of the subject does not receive rescue treatment with a VEGF inhibitor to arrest or reverse progression of the ocular neovascularization during the period between about 180 days and about one year following administration of the pharmaceutical composition.

[0003b] In another aspect of the present disclosure, there is provided a use of an rAAV comprising a nucleic acid sequence encoding an anti-VEGF protein in the manufacture of a pharmaceutical composition for the treatment of ocular neovascularization in an eye of a human subject suffering from ocular neovascularization, said eye of the human subject having previously received at least one dose of a VEGF inhibitor, wherein a unit dose of the pharmaceutical composition comprises at least 1×10^6 and at most 1×10^{15} vector genomes of the rAAV and a pharmaceutically acceptable carrier, and wherein one or two doses of a VEGF inhibitor are to be administered to the eye of the subject in a 30 day period after the

pharmaceutical composition is to be administered.

[0004] The present disclosure provides compositions and methods for treating CNV, such as found in the wet form of AMD, in a human subject.

[0005] In one example, the present disclosure provides compositions and methods for treating AMD in a human subject, comprising: administering subretinally a pharmaceutical composition comprising a pharmaceutically effective amount of a VEGF inhibitor to a human subject in need of treatment for AMD. In one example, the pharmaceutical composition comprises a recombinant virus. In another example, the VEGF inhibitor comprises a nucleic acid encoding soluble Fms-related tyrosine kinase-1 (sFLT-1) protein.

[0006] In one example, the present disclosure provides compositions and methods for the prevention of CNV in human subjects with AMD, comprising: administering subretinally a pharmaceutical composition comprising a pharmaceutically effective amount of a recombinant virus comprising a nucleic acid encoding soluble Fms-related tyrosine kinase-1 (sFLT-1) protein to a human subject in need of a treatment for AMD.

[0007] In some examples, the virus is selected from adeno-associated virus (AAV), helper-dependent adenovirus, retrovirus, herpes simplex virus, lentivirus, poxvirus, hemagglutinatin virus of Japan-liposome (HVJ) complex, Moloney murine leukemia virus, and HIV-based virus. In some examples, the AAV capsid or inverted terminal repeats (ITRs) is selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, rh10, and hybrids thereof.

[0008] In some examples, the recombinant virus comprises a promoter selected from cytomegalovirus (CMV) promoter, Rous sarcoma virus (RSV) promoter, MMT promoter,

EF-1 alpha promoter, UB6 promoter, chicken beta-actin promoter, CAG promoter, RPE65 promoter and opsin promoter.

[0009] In some examples, the recombinant virus comprises an enhancer.

[0010] In some examples, the recombinant virus comprises an intron or chimeric intron.

[0011] In some examples, the recombinant virus comprises a SV40 poly A sequence.

[0012] In some examples, the recombinant virus comprises a human sFlt-1 protein or a functional fragment thereof.

[0013] In some examples, the recombinant virus is generated from a plasmid comprising either an ampicillin resistance marker or a non-ampicillin resistance marker.

[0014] In some examples, the recombinant virus comprises bacterial regulatory sequences such as a T7 RNA polymerase promoter.

[0015] In some examples, the recombinant virus lacks bacterial regulatory sequences such as a T7 RNA polymerase promoter.

[0016] In some examples, the recombinant virus comprises a regulatory nucleic acid fragment that is capable of directing selective expression of the sFlt-1 protein or a functional fragment thereof in an eye cell.

[0017] In some examples, the pharmaceutical composition comprises about 1×10^6 to about 1×10^{15} recombinant viral vector genomes, about 1×10^7 to about 1×10^{14} recombinant viral vector genomes, about 1×10^8 to about 1×10^{13} recombinant viral vector genomes, about 1×10^9 to about 3×10^{12} recombinant viral vector genomes, or about 1×10^{10} to about 3×10^{12} recombinant viral vector genomes.

[0018] In some examples, the pharmaceutical composition is administered via subretinal injection.

[0019] In some examples, the method further comprises administering to the human subject a pharmaceutically effective amount of a VEGF inhibitor. In some examples, the VEGF inhibitor comprises an antibody against VEGF or a functional fragment thereof. In some examples, the VEGF inhibitor comprises ranibizumab. In some examples, the pharmaceutical composition is administered at least 5, 6, 7, or 8 days after the administering the VEGF inhibitor. In some examples, the pharmaceutical composition is administered within 30, 60, or 90 days of administering the VEGF inhibitor.

[0020] In some examples, the VEGF inhibitor is administered for 1 time prior to administering the pharmaceutical composition comprising the recombinant virus and 1 to 2 times following administration. In some examples, the VEGF inhibitor is administered for

at least 2 times prior to administering the pharmaceutical composition and 1 to 2 times following administration. In some examples, the VEGF inhibitor is administered over a period of 6 to 7 weeks.

[0021] In some examples the VEGF inhibitor is an anti-VEGF antibody, such as bevacizumab or ranibizumab. In other examples the VEGF inhibitor is a soluble receptor, fusion protein, or fragment thereof, such as aflibercept or sFLT01.

[0022] In some examples, the AMD is wet AMD.

[0023] In some examples, AMD is dry AMD.

[0024] In some examples, the human subject is at risk for wet AMD.

[0025] In some examples, the human subject presents symptoms of early stage wet AMD.

[0026] In some examples, at least 3, 5, 10, 15, or 20 treatments of a different VEGF inhibitor for the treatment of AMD have been previously administered to said human subject

[0027] In some examples, best corrected visual acuity (BCVA) did not improve after said treatment with ranibizumab.

[0028] In some examples, best corrected visual acuity (BCVA), as measured by ETDRS (Early Treatment Diabetic Retinopathy Study) letters, improves by more than 1 line after said treatment with ranibizumab.

[0029] In some examples, human subject presents symptoms of early stage dry AMD.

[0030] In some examples, treatment is administered at a frequency of at least biannually.

[0031] In some examples, administering step is carried out in said human subject where the subject is age 20, 40, 50, 55, or 65 years or older.

[0032] In some examples, administration is to a site outside the fovea.

[0033] In some examples, administration is to one or more cells of the subretinal space of the central retina.

[0034] In some examples, administration is to one or more cells of the outer macula.

[0035] In some examples, administration is to one or more cells of the inner macula.

[0036] In some examples, administration is to retinal pigment epithelial cells.

[0037] In some examples, administration does not adversely affect central retinal function or central retinal structure.

[0038] In some examples, administration does not increase systemic levels of VEGF inhibitor in the human subject.

[0039] In some examples, administration does not increase systemic levels of sFlt-1 in the human subject.

[0040] In some examples, administering step is carried out simultaneously, or sequentially in both eyes

[0041] In some examples, administering step is carried out in one eye.

[0042] In some examples, administering step is carried out in one eye when fellow eye presents symptoms of AMD.

[0043] In some examples, administering step is carried out in a human subject resistant to penicillin.

[0044] In some examples, administering step is carried out in a human subject sensitive to penicillin.

[0045] In some examples, administering step is carried out in a human subject allergic to penicillin.

[0046] In some examples, administering step is carried out in a human subject not allergic to penicillin.

[0047] In some examples, administering step causes no inflammation of the vitreous is observed by biomicroscopy (BE) and indirect opthalmoscopy (IOE) following the administering step.

[0048] In some examples, administering step does not cause a cytotoxic T cell.

[0049] In some examples, administering step does not cause a cytotoxic T cell response a measure by in increase in cytotoxic T cells of less than 10% greater than the baseline range.

[0050] In some examples, T cells do not display an activated effector phenotype following the administering step.

[0051] In some examples, best corrected visual acuity (BCVA) improves by 1, 2, 3, 4 or 5 lines or more. as measured by ETDRS (Early Treatment Diabetic Retinopathy Study) letters, following the administering step.

[0052] In some examples, reduction in neovascularization is observed using Fluorscein Angiography (FA) following the administering step

[0053] In some examples, frequency of administration of ranibizumab is reduced to less than 12 doses per year. In some examples, frequency of administration of aflibercept is reduced to less than 6 doses per year.

[0054] In some examples, ranibizumab or aflibercept or other VEGF inhibitor is administered with reduced frequency or no longer administered.

[0055] In some examples, the virus comprises a sFLT-1 gene or a functional fragment thereof with ≥90% sequence homology to the human sFLT-1 gene sequence.

[0056] In some examples, the virus administered comprises a sFLT-1 gene, gene variant or gene fragment.

[0057] In some examples, no vector is detected in the human subject's tear, blood, saliva or urine samples 7, 14, 21 or 30 days after administering the pharmaceutical composition.

[0058] In some examples, the presence of the viral vector is detected by qPCR or ELISA.

[0059] In some examples, the sFLT-1 protein levels in the vitreous of the human subject is about 500 – 5,000 pg/ml, about 600 – 4,000 pg/ml, about 800 – 3,000 pg/ml about 900 – 2,000 pg/ml, or about 1,000 – 1,800 pg/ml 7, 14, 21 or 30 days after administering the pharmaceutical composition. In some examples, the sFlt-1 protein level, which may also be called the sFlt-1 protein concentration, in the vitreous of the human subject is elevated at 7, 14, 31, 30, 60, 90, 180, 270 and 365 days after administering the pharmaceutical composition.

[0060] In some examples, the human subject shows no clinically significant retinal toxicity as assessed by serial ophthalmic examinations over least a two months period.

[0061] In some examples, no superficial, anterior segment or vitreous inflammatory signs are present in the human subject over least a two months period.

[0062] In some examples, the human subject does not require rescue treatment with a VEGF inhibitor at least 120 days post administering the recombinant viruses. In some examples, the human subject does not require rescue treatment with a VEGF inhibitor at least 180 days or at least 210 days post administering the recombinant viruses. In some examples, the human subject does not require rescue treatment with a VEGF inhibitor for at least 270 days after administering the recombinant viruses. In some examples, the human subject does not require rescue treatment with a VEGF inhibitor for at least 365 days after administering the recombinant viruses.

[0063] In some examples, there is no evidence of visual acuity loss, IOP elevation, retinal detachment, or any intraocular or systemic immune response in said human subject at least 180 days or at least 210 days post said administering the recombinant viruses. In some examples, there is no evidence of visual acuity loss, IOP elevation, retinal detachment,

or any intraocular or systemic immune response in said human subject at least 365 days after administering the recombinant viruses.

[0064] In another example, the present disclosure provides a pharmaceutical composition comprising about 1 x 10^6 to about 1 x 10^{15} recombinant viruses, wherein each of the recombinant virus comprises a nucleic acid encoding soluble Fms-related tyrosine kinase-1 (sFlt-1) protein.

[0065] In some examples, the present disclosure provides for a method for the treatment or prophylaxis of ocular neovascularization in a human subject comprising: administering to one or more subretinal sites a pharmaceutically effective amount of a pharmaceutical composition comprising a nucleic acid encoding sFLT-1 to a human subject in need of treatment.

[0066] In some examples, the present disclosure provides for a human subject that has or is suspected of having one or more conditions selected from the group consisting of: age-related macular degeneration (AMD), wet-AMD, dry-AMD, retinal neovascularization, choroidal neovascularization and diabetic retinopathy. In some cases the human subject has or is suspected of having one or more conditions selected from the group consisting of: proliferative diabetic retinopathy, retinal vein occlusion, central retinal vein occlusion, branched retinal vein occlusion, diabetic macular edema, diabetic retinal ischemia, ischemic retinopathy and diabetic retinal edema.

[0067] In some examples, the present disclosure provides for a pharmaceutical composition comprising a recombinant virus, the virus selected from the group consisting of: adeno-associated virus (AAV), adenovirus, helper-dependent adenovirus, retrovirus, herpes simplex virus, lentivirus, poxvirus, hemagglutinatin virus of Japan-liposome (HVJ) complex, Moloney murine leukemia virus, and HIV-based virus.

[0068] In some examples, the present disclosure provides for a nucleic acid encoding the sFLT-1 which is operatively linked to a promoter selected from the group consisting of: cytomegalovirus (CMV) promoter, Rous sarcoma virus (RSV) promoter, MMT promoter, EF-1 alpha promoter, UB6 promoter, chicken beta-actin promoter, CAG promoter, RPE65 promoter and opsin promoter.

[0069] In some examples, the present disclosure provides sFLT-1 nucleic acid, wherein the sFLT-1 encodes at least 1 dimerization domain. In some cases the sFLT-1 nucleic acid does not contain a prokaryotic regulatory sequence. In some cases the sFLT-1 nucleic acid does contain a prokaryotic regulatory sequence.

[0070] In some examples, the present disclosure provides for a pharmaceutical composition comprising a virus or a plasmid.

[0071] In some examples, the present disclosure provides for administration of one or more treatments of a VEGF inhibitor to the human subject. In some cases the VEGF inhibitor is administered within 30, 90, or 180 days of administration of the pharmaceutical composition. In some cases the pharmaceutical composition of the present disclosure and VEGF inhibitor are administered at least 24 hours apart.

[0072] In some examples, the present disclosure provides for a pharmaceutical composition administered to a human subject at least 55 years old.

[0073] In some examples, the present disclosure provides for administering the pharmaceutical composition outside the fovea.

[0074] In some examples, the present disclosure provides for the best corrected visual acuity (BCVA) of the human subject, to improve by at least 1, 2, 3, 4 or 5 lines as measured by ETDRS (Early Treatment Diabetic Retinopathy Study) letters following the administering of the pharmaceutical composition.

[0075] In some examples, the present disclosure provides for the best corrected visual acuity (BCVA) to decrease by fewer than 15 letters as measured by ETDRS (Early Treatment Diabetic Retinopathy Study) following the administering of the pharmaceutical composition.

[0076] In some examples, the present disclosure provides for administering the pharmaceutical composition under conditions selected from the group consisting of: administering the pharmaceutical composition in one eye, administering the pharmaceutical composition sequentially in two eyes, and administering the pharmaceutical composition simultaneously in two eyes.

[0077] In some examples, the present disclosure provides for a reduction in neovascularization as observed by a Fluorscein Angiography (FA) follows the administering of the pharmaceutical composition.

[0078] In some examples, the present disclosure provides for no superficial, anterior segment or vitreous inflammatory signs are present in the human subject at least 1 week after injection.

[0079] In some examples, the present disclosure provides for no superficial, anterior segment or vitreous inflammatory signs are present in the human subject at 1 week or at 3, 6, 9 or 12 months after administration of the pharmaceutical composition.

[0080] In some examples, the present disclosure provides for the human subject not to require rescue treatment for at least 30, 60, 90, 120, 180, 270 or 365 days after the administering of the pharmaceutical composition.

[0081] In some examples, the present disclosure provides for the human subject to experience no visual acuity loss, IOP elevation, retinal detachment, intraocular or systemic immune response after administering the pharmaceutical composition.

[0082] In some examples, the present disclosure provides for no increased anti-AAV cytotoxic T cell response is measured following the administering step.

[0083] In some examples, the present disclosure provides for no virus detected in the human subject's blood, saliva or urine samples, 3, 7, 14, 21 or 30 days after administering the pharmaceutical composition.

[0084] In some examples, the present disclosure provides for sFLT-1 protein levels in the vitreous of the human subject to be about 500 – 5,000 pg/ml, 7, 14, 21, 30, 60, 90, 120, 150, 180, 270 or 365 days after administering the pharmaceutical composition in the human subject.

[0085] In some examples, the present disclosure provides for the human subject to receive one or more treatments with VEGF inhibitors prior to the administering of the pharmaceutical composition.

[0086] In some examples, the present disclosure provides for the human subject as resistant to treatment with VEGF inhibitors.

[0087] In some examples, the present disclosure provides for a human subject who has not previously received a VEGF inhibitor before administering the pharmaceutical composition.

[0088] In some examples, the present disclosure provides for administering of the pharmaceutical composition at a frequency less than 3 times a year in the human subject.

[0089] In some examples, the present disclosure provides for administering of the pharmaceutical composition to reduce the frequency of administration of additional VEGF inhibitor treatments in the human subject.

[0090] In some examples, the present disclosure provides for the concentration of sFLT-1 protein in the vitreous of the human subject to be elevated when measured at 7, 14, 21, 30, 60, 90, 120, 150, 180, 270 or 365 days after administering of the pharmaceutical composition.

[0091] In some examples, the present disclosure provides for a human subject who has the vitreous gel removed prior to or within one day or one week of the administration of the pharmaceutical composition.

[0092] In some examples, the present disclosure provides for a pharmaceutical composition administered using a vitrectomy system that is smaller than 20 gauge.

[0093] In some examples, the present disclosure provides for a pharmaceutical composition administered using a vitrectomy system that does not require sutures.

[0094] In some examples, the present disclosure provides for a pharmaceutical composition administered using a cannula tip that is smaller than 39 gauge.

[0095] In some examples, the present disclosure provides for a pharmaceutical composition followed by gas/fluid exchange in the vitreous chamber.

[0096] In some examples, the present disclosure provides for the central retinal thickness of the subject not to increase by more than 50 microns, 100 microns, or 250 microns within 12 months following treatment with said pharmacological agent.

[0097] In some examples, the present disclosure provides for geographic atrophy not to progress in the diseased eye of the human subject as compared to the diseased eyes of untreated human subjects.

[0098] In some examples, the present disclosure provides for a pharmaceutical composition comprising recombinant viruses or plasmids comprising a nucleic acid comprising at least 1 promoter sequence operatively linked to a sFLT-1 transgene sequence. In some cases the pharmaceutical composition of the present disclosure comprises a promoter sequence and the sFLT-1 transgene sequence separated by a sequence greater than 300 base pairs. In some cases the pharmaceutical composition of the present disclosure comprises a promoter sequence and the sFLT-1 transgene sequence separated by a UTR sequence. In some cases the UTR sequence comprises at least 10 base pairs. In some cases, the pharmaceutical composition comprises at least 3 linker sequences each comprising at least 50 base pairs.

[0099] In some examples, the present disclosure provides for a pharmaceutical composition, wherein the sFLT-1 nucleic acid encodes at least 1 dimerization domain.

[00100] In some examples, the present disclosure provides for a pharmaceutical composition comprising a promoter sequence selected from the group consisting of SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19, SEQ ID No. 20, SEQ ID No. 21, SEQ ID No. 22, SEQ ID No. 23, SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, SEQ ID No. 27, SEQ ID No. 28, SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 31, SEQ ID No. 32, SEQ ID No. 33, SEQ ID No. 34, SEQ ID No. 35, SEQ ID No. 36, SEQ ID No. 37, SEQ ID No. 38, SEQ ID No. 39, SEQ ID No. 340, SEQ ID No. 41, SEQ ID No. 42, SEQ ID No. 43, SEQ ID No. 44, SEQ ID No. 45, SEQ ID No. 46, and SEQ ID No. 47; a sequence encoding a

VEGF inhibitor selected from the group consisting of SEQ ID No. 102, SEQ ID No. 103, SEQ ID No. 104, SEQ ID No. 105, SEQ ID No. 106, SEQ ID No. 107 and SEQ ID No. 108; an intron sequence consisting of SEQ ID No. 48, SEQ ID No. 115, SEQ ID No. 116, SEQ ID No. 117, SEQ ID No. 118, and SEQ ID No. 119; a UTR sequence selected from the group consisting of SEQ ID No. 91, SEQ ID No. 2, SEQ ID No. 92, SEQ ID No. 93, SEQ ID No. 94, SEQ ID No. 95, SEQ ID No. 96, SEQ ID No. 97, SEQ ID No. 98, SEQ ID No. 99, SEQ ID No.100, and SEQ ID No. 101; and a termination sequence selected from the group consisting of SEQ ID No. 49, SEQ ID No. 50, SEQ ID No. 51, SEQ ID No. 52, SEQ ID No. 53, SEQ ID No. 54, and SEQ ID No. 55.

[00101] In some examples, the present disclosure provides for a unit dose of a pharmaceutical composition comprising recombinant viruses of $1x10^6$ to $1x10^{15}$ vector genomes, wherein the recombinant viruses comprise a nucleic acid encoding sFLT-1 operatively linked to a promoter. In some cases the unit dose of the pharmaceutical composition comprises $1x10^{10}$ to $3x10^{12}$ vector genomes.

[00102] In some examples, the present disclosure provides for a method of generating a recombinant virus in a cell, the method comprising: introducing into a cell, a nucleic acid comprising at least 1 promoter sequence operatively linked to an sFLT-1 transgene sequence, an ITR sequence, and UTR sequence; and purifying the recombinant virus. In some cases the UTR sequence is a human UTR sequence. In some cases, the nucleic acid sequence does not contain a beta-lactam antibiotic resistance sequence. In some cases the recombinant virus produces sFLT-1 protein in the range of 100-10,000 pg/mL when measured at 72 hours following transduction of HEK293 cells at a multiplicity of infection (MOI) of $1x10^6$. In some cases, the recombinant virus inhibits proliferation of human umbilical vascular endothelial (HUVEC) cells.

[00103] In some examples, the present disclosure provides for a cell for generating recombinant viral vector, the cell comprising at least 1 promoter polynucleotide sequence operatively linked to a sFLT-1 transgene sequence, an ITR polynucleotide sequence, and a UTR polynucleotide sequence.

[00104] In some examples, the present disclosure provides for a nucleic acid comprising a sequence encoding sFLT-1 for use in treatment or prophylaxis of ocular neovascularization in a human; wherein said use comprises administering directly to a human subject in need thereof, to one or more sub retinal sites in said human subject, an effective amount of a

pharmaceutical composition; wherein said pharmaceutical composition comprises said nucleic acid.

[00105] In some examples, the present disclosure provides the nucleic acid for use, wherein said sFLT-1 is an inhibitor of VEGF and wherein said treating or reducing the likelihood of ocular neovascularization occurs as a result of VEGF inhibition.

[00106] In some examples, the present disclosure provides for the nucleic acid for use, wherein the pharmaceutical composition is capable of elevating levels of sFLT-1 protein in the vitreous of the human subject after at least 72 hours after administration of said pharmaceutical composition to said human subject, compared to levels of sFLT-1 protein in the vitreous of said human prior to said administration.

[00107] In some examples, the present disclosure provides for the nucleic acid for use, wherein the nucleic acid comprising said sFLT-1 comprises a recombinant virus, the virus selected from the group consisting of: adeno-associated virus (AAV), adenovirus, helper-dependent adenovirus, retrovirus, herpes simplex virus, lentivirus, poxvirus, hemagglutinatin virus of Japan-liposome (HVJ) complex, Moloney murine leukemia virus, and HIV-based virus.

[00108] In some examples, the present disclosure provides for the nucleic acid for use, wherein the nucleic acid encoding the sFLT-1 is operatively linked to a promoter selected from the group consisting of: cytomegalovirus (CMV) promoter, Rous sarcoma virus (RSV) promoter, MMT promoter, EF-1 alpha promoter, UB6 promoter, chicken beta-actin promoter, CAG promoter, RPE65 promoter and opsin promoter.

[00109] In some examples, the present disclosure provides for the nucleic acid for use, wherein the nucleic acid is packaged by a virus or is plasmid DNA.

[00110] In some examples, the present disclosure provides for the nucleic acid for use, said use further comprising administration of one or more additional VEGF inhibitors to the human subject in need of treatment or reduction, optionally wherein said additional VEGF inhibitor is ranibizumab or bevacizumab.

[00111] In some examples, the present disclosure provides for the nucleic acid for use, said use comprising administering said pharmaceutical composition to a human subject at least 50, 55, or 65 years old.

[00112] In some examples, the present disclosure provides for the nucleic acid for use, said use comprising administering said pharmaceutical composition outside the fovea.

[00113] In some examples, the present disclosure provides for the nucleic acid for use, wherein the best corrected visual acuity (BCVA) of the human subject in need of treatment, improves by at least 1, 2, 3, 4 or 5 lines as measured by ETDRS (Early Treatment Diabetic Retinopathy Study) letters following the administering of an effective amount of the pharmaceutical composition.

[00114] In some examples, the present disclosure provides for the nucleic acid for use, wherein the administering of the pharmaceutical composition is performed at a frequency at least once per 3, 6, 9, 12, 18, or 24 months in a human subject in need of treatment.

[00115] In some examples, the present disclosure provides for the nucleic acid for use, wherein the administering of the pharmaceutical composition is performed at a frequency less than 3 times a year in the human subject or is performed at a frequency reducing the frequency of administration of additional VEGF inhibitor treatments in the human subject.

[00116] In some examples, the present disclosure provides for a unit dose of pharmaceutical composition comprising about $1x10^6$ to $1x10^{15}$ or $1x10^{10}$ to $3x10^{12}$ vector genomes. In some examples, the recombinant viruses comprise a nucleic acid encoding sFLT-1, or a functional fragment thereof, operatively linked to a promoter.

[00117] In some examples, the present disclosure provides for a method for the treatment or prophylaxis of ocular neovascularization in a human subject comprising: administering to one or more subretinal sites a pharmaceutically effective amount of a pharmaceutical composition comprising a nucleic acid encoding a VEGF inhibitor to a human subject in need of treatment. In some examples, the VEGF inhibitor is an anti-VEGF antibody or a functional fragment thereof. In some examples, the VEGF inhibitor is a soluble receptor, fusion protein, or a functional fragment thereof.

INCORPORATION BY REFERENCE

[0002] 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[00118] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets

CLAIMS

WHAT IS CLAIMED IS:

1. Use of an rAAV comprising a nucleic acid sequence encoding an anti-VEGF protein in the manufacture of a pharmaceutical composition for the treatment of ocular neovascularization in an eye of a human subject suffering from ocular neovascularization, said eye of the human subject having previously received a first intravitreal injection of a VEGF inhibitor in an amount such that the VEGF inhibitor is present in the eye of the subject at a concentration sufficient to prevent progression of the neovascularization at the time of administration of the pharmaceutical composition, wherein a unit dose of the pharmaceutical composition comprises at least 1×10^6 and at most 1×10^{15} vector genomes of the rAAV and a pharmaceutically acceptable carrier and is sufficient to cause elevated levels of said anti-VEGF protein in the eye of said human subject when measured at least one year after administration of the pharmaceutical composition, and wherein the eye of the subject does not receive rescue treatment with a VEGF inhibitor to arrest or reverse progression of the ocular neovascularization during the period between about 180 days and about one year following administration of the pharmaceutical composition.
2. Use of an rAAV comprising a nucleic acid sequence encoding an anti-VEGF protein in the manufacture of a pharmaceutical composition for the treatment of ocular neovascularization in an eye of a human subject suffering from ocular neovascularization, said eye of the human subject having previously received at least one dose of a VEGF inhibitor, wherein a unit dose of the pharmaceutical composition comprises at least 1×10^6 and at most 1×10^{15} vector genomes of the rAAV and a pharmaceutically acceptable carrier, and wherein one or two doses of a VEGF inhibitor are to be administered to the eye of the subject in a 30 day period after the pharmaceutical composition is to be administered.
3. The use of claim 2, wherein the eye of the subject does not receive rescue treatment with a VEGF inhibitor to arrest or reverse progression of the ocular neovascularization during the period between about 180 days and about 365 days following the administration of the unit dose.
4. The use of any one of claims 1 to 3, wherein the rAAV is selected from the group

consisting of: AAV1, AAV2, AAV2.5 AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12.

5. The use of claim 4, wherein the rAAV is selected from the group consisting of: AAV2 and AAV8.
6. The use of any one of claims 1 to 5, wherein the unit dose comprises 1 x 10^9 and at most 3x10^{13} vector genomes of the rAAV.
7. The use of any one of claims 1 to 6, wherein the eye of the subject is to receive the first intravitreal injection of a VEGF inhibitor between about 1 to 30 days prior to the administration of the pharmaceutical composition.
8. The use of any one of claims 1 to 7, wherein the VEGF inhibitor is aflibercept.
9. The use of any one of claims 1 to 7, wherein the VEGF inhibitor is ranibizumab.
10. The use of any one of claims 1 to 9, wherein the anti-VEGF protein comprises a functional fragment of human sFLTl having at least 90% sequence identity to SEQ ID NO: 121.
11. The use of claim 10, wherein the anti-VEGF protein is sFLTl.
12. The use of claim 10, wherein the anti-VEGF protein is aflibercept.
13. The use of any one of claims 1 to 12, wherein the eye of the subject does not receive rescue treatment with a VEGF inhibitor to arrest or reverse progression of the ocular neovascularization during the period between about 90 days and about one year following the administration of the pharmaceutical composition.
14. The use of any one of claims 1 to 13, wherein the pharmaceutical composition is to be administered to the subject, and subsequently visual acuity in the eye of the subject by Early Treatment Diabetic Retinopathy Study (ETDRS) is measured, wherein the best corrected visual acuity (BCVA) of the human subject improves by at least 1 line as measured by ETDRS letters following the administering of the pharmaceutical composition.