TREATMENT OF AMD USING AAV SFLT-1

SEQUENCE LISTING

[0001] This application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 1, 2013, is named 43016-702.201_SL.txt and is 84,779 bytes in size.

BACKGROUND OF THE DISCLOSURE

[0002] Age-related macular degeneration (AMD) is one of the leading causes of vision irreversible damage in people over the age of 50 years. AMD is clinically divided into two types as "dry" and "wet". The wet form of AMD may develop rapidly and often results in blindness. The pathological changes of the disease may cause severe visual impairment. The manifestations of AMD may include, but is not limited to retinal pigment epithelial cells (RPE) dysfunction and choroidal neovascularization (CNV) in the macular area. Fluid leakage, RPE or neural epithelial detachment and bleeding from ruptured blood vessels can occur in severe cases. It has been found that many cellular factors play important roles in regulation in CNV generation, among which may include but are not limited to vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR), platelet-derived growth factor (PDGF), hypoxia inducible factor (HIF), angiopoietin (Ang) and other cytokines, mitogen-activated protein kinases (MAPK) and others.

[0003] One currently approved treatment for wet AMD is Lucentis®. Lucentis® is an anti-angiogenesis agent and targets all isoforms of Vascular Endothelial Growth Factor (VEGF). Clinical studies have shown improved or stable vision in approximately 95% of patients administered Lucentis®, compared to approximately 60% of the patients who received sham treatment. Although Lucentis® is the first approved agent to improve vision it requires intravitreal administrations every 4 weeks for optimal visual benefit. Eylea® is another VEGF inhibitor that has been approved to treat wet AMD. Eylea® also requires frequent intravitreal injections every 4-8 weeks for optimal visual benefit. Intravitreal routes of administration may increase risks for serious complications such as infectious endophthalmitis and retinal detachment, for which cumulative risk increases with repeated administrations. Increased intraocular pressure, traumatic cataract, and retinal tears have also been reported. Finally, with a treatment that is delivered by an ophthalmologist, treatment frequency determines the burden to the patient, physician, and

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

[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 aspect, 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 aspect, the pharmaceutical composition comprises a recombinant virus. In another aspect, the VEGF inhibitor comprises a nucleic acid encoding soluble Fms-related tyrosine kinase-1 (sFLT-1) protein.

[0006] In one aspect, 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 aspects, 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 aspects, 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 aspects, 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 aspects, the recombinant virus comprises an enhancer.

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

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

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

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

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

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

[0016] In some aspects, 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 aspects, 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 aspects, the pharmaceutical composition is administered via subretinal injection.

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

[0020] In some aspects, 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 aspects, 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 aspects, the VEGF inhibitor is administered over a period of 6 to 7 weeks.

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

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

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

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

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

[0026] In some aspects, 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 aspects, best corrected visual acuity (BCVA) did not improve after said treatment with ranibizumab.

[0028] In some aspects, 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 aspects, human subject presents symptoms of early stage dry AMD.

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

[0031] In some aspects, 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 aspects, administration is to a site outside the fovea.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[0049] In some aspects, 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 aspects, T cells do not display an activated effector phenotype following the administering step.

[0051] In some aspects, 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 aspects, reduction in neovascularization is observed using Fluorscein Angiography (FA) following the administering step

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

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

[0055] In some aspects, 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 aspects, the virus administered comprises a sFLT-1 gene, gene variant or gene fragment.

[0057] In some aspects, 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 aspects, the presence of the viral vector is detected by qPCR or ELISA.

[0059] In some aspects, 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 aspects, 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 aspects, the human subject shows no clinically significant retinal toxicity as assessed by serial ophthalmic examinations over least a two months period.

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

[0062] In some aspects, the human subject does not require rescue treatment with a VEGF inhibitor at least 120 days post administering the recombinant viruses. In some aspects, 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 aspects, the human subject does not require rescue treatment with a VEGF inhibitor for at least 270 days after administering the recombinant viruses. In some aspects, 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 aspects, 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 aspects, 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 aspect, 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 aspects, the 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 aspects, the 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 aspects, the 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 aspects, the 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 aspects, the 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 aspects, the disclosure provides for a pharmaceutical composition comprising a virus or a plasmid.

[0071] In some aspects, the 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 disclosure and VEGF inhibitor are administered at least 24 hours apart.

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

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

[0074] In some aspects, the 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 aspects, the 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 aspects, the 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 aspects, the disclosure provides for a reduction in neovascularization as observed by a Fluorscein Angiography (FA) follows the administering of the pharmaceutical composition.

[0078] In some aspects, the 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 aspects, the 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 aspects, the 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 aspects, the 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 aspects, the disclosure provides for no increased anti-AAV cytotoxic T cell response is measured following the administering step.

[0083] In some aspects, the 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 aspects, the 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 aspects, the 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 aspects, the disclosure provides for the human subject as resistant to treatment with VEGF inhibitors.

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

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

[0089] In some aspects, the 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 aspects, the 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 aspects, the 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 aspects, the disclosure provides for a pharmaceutical composition administered using a vitrectomy system that is smaller than 20 gauge.

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

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

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

[0096] In some aspects, the 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 aspects, the 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 aspects, the 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 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 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 aspects, the disclosure provides for a pharmaceutical composition, wherein the sFLT-1 nucleic acid encodes at least 1 dimerization domain.

[00100] In some aspects, the 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 aspects, the 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 aspects, the 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 aspects, the 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 aspects, the 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 aspects, the 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 aspects, the 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 aspects, the 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 aspects, the 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 aspects, the disclosure provides for the nucleic acid for use, wherein the nucleic acid is packaged by a virus or is plasmid DNA.

[00110] In some aspects, the 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 aspects, the 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 aspects, the disclosure provides for the nucleic acid for use, said use comprising administering said pharmaceutical composition outside the fovea.

[00113] In some aspects, the 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 aspects, the 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 aspects, the 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 aspects, the 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 aspects, the recombinant viruses comprise a nucleic acid encoding sFLT-1, or a functional fragment thereof, operatively linked to a promoter.

[00117] In some aspects, the 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 aspects, the VEGF inhibitor is an anti-VEGF antibody or a functional fragment thereof. In some aspects, the VEGF inhibitor is a soluble receptor, fusion protein, or a functional fragment thereof.

INCORPORATION BY REFERENCE

[00118] 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

[00119] 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

forth illustrative aspects, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[00120] FIG. 1 depicts the schematic representation of an exemplary plasmid.

[00121] FIG. 2 depicts expression, secretion and biological activity of sFLT-1 from rAAV.sflt-1-transduced cells. **(a)** Western blot analysis of conditioned media from Ad.sFlt-1-transduced 293 cells (lane 1), rAAV.sFlt-1-transduced D407 cells (lane 2), rAAV.sFlt-1-transduced 293 cells (lane 3), and AAV.gfp-transduced D407 cells (lane 4). **(b)** Inhibition of VEGF-induced HUVEC proliferation by conditioned media from rAAV.sFlt-1-transduced cells. HUVECs were cultured in starvation medium (column 1), in medium containing recombinant VEGF (column 2), in medium containing VEGF and 40 μL conditioned medium from rAAV.sFlt-1-transduced 293 cells (column 3), in medium containing VEGF and 80 μL conditioned medium from rAAV.sFlt-1-transduced 293 cells (column 4), and in medium containing VEGF and 80 μL conditioned medium from rAAV.gfp-transduced 293 cells (column 5). (*P < 0.02, **P <0.005 for differences between rAAV.sFlt-1 plus VEGF, and VEGF only.

[00122] FIG. 3A depicts graph showing human sFlt-1 (hsFLT-1) expression in the vitreous of monkeys injected in the left eyes with rAAV.sFlt-1 (Monkey 8514, 8530, 8523, 8524 and 999), rAAV.gfp (Monkey 8297 and 8532), in both eyes with recombinant sFLT-1 protein (Monkey 8294) and control uninjected monkey (control). Control and monkeys 8294 and 999 were euthanized at 3 months post injection, Monkey 8524 was euthanized at 9 months post injection and monkeys 8297, 8532, 8514, 8530 and 8523 were euthanized at 12 months post injection. * denotes sFLT-1 protein levels that are significantly higher in the rAAV.sFlt-1 injected eyes ($p < 0.05$). **FIG. 3B** depicts graphs showing hsFLT-1 levels in the rAAV.sFlt-1-injected (999, 8524, 8523, 8530 and 8514), rAAV.gfp-injected (8297 and 8532), recombinant sFlt-1 protein-injected (8294) and uninjected (control) monkeys at different times post injection.

[00123] FIG. 4: Immune Cell Subset Population in mouse eyes. Graphs showing immune cell subset population at the different times post injection.

[00124] FIG. 5: Immune Cell Subset Population in mouse spleens. Graphs showing immune cell subset population at the different times post injection.

[00125] FIG. 6 depicts diagrams comparing Ki67 responses in CD4+ T cells in different mice at different times post injection.

[00126] FIGS. 7A to **7D** depict various exemplary replication origin sequences.

[00127] **FIGS. 8A** to **8F** depict the sequences of various exemplary promoters.

[00128] **FIGS. 9A** to **9C** depict the sequence of various exemplary introns, poly A sequences, and ITR regions.

[00129] **FIGS. 9D** to **9F** depict the sequence of various exemplary linker sequences.

[00130] **FIGS. 9G** to **9H** depict the sequence of various exemplary UTR sequences.

[00131] **FIGS. 10A** to **10C** depict the sequence encoding various exemplary anti-VEGF proteins.

[00132] **FIG. 11A** depicts the amino acid sequence of sFLT-1. **FIG 11B** depicts the amino acid sequence of sFLT-1 domain 2, a functional fragment of sFLT-1. **FIG 11C** depicts a nucleic acid sequence coding for sFLT-1 domain 2.

[00133] **FIGS. 12A** to **12B** depict the sequences of various exemplary antibiotic resistance genes.

[00134] **FIG. 13** depicts the PK of one exemplary composition (rAAV.sFlt-1), wherein it reaches optimal anti-VEGF expression at 6-8 weeks. RBZ is a standard care of anti-VEGF, such as ranibizumab. "RBZ rescue" means rescue treatment.

[00135] **FIG. 14** depicts ophthalmologic assessment of the patients. Inflammation was evaluated by biomicroscopy (BE) and indirect ophthalmoscopy (IOE). Unrem: unremarkable.

[00136] **FIGS. 15A** and **15B** depict visual acuity results.

[00137] **FIG. 16** depicts the measurement of retina thickness of a patient who was given 24 previous Lucentis injections.

[00138] **FIG. 17** depicts biodistribution: qPCR for sFLT-1 sequence (copy number detected).

[00139] **FIG. 18** depicts biodistribution: AAV capsid measured by ELISA, AAV titer in capsids/mL.

[00140] **FIG. 19** depicts biodistribution of sFLT-1 measured by ELISA. Shown are human sFLT-1 concentration (pg/mL).

[00141] **FIGS. 20A** and **20B** depict OCT assessments of patients administered with either low dose rAAV.sFlt-1 (R1, R2, R4) or high dose of rAAV.sFlt-1 (R5, R6 and R8).

[00142] **FIGS. 21A** and **21B** depict visual acuity results of human subjects treated with rAAV.sFlt-1 vs. untreated control patients at 180 days following treatment.

[00143] **FIGS. 22A** and **22B** depict visual acuity results of human subjects treated with rAAV.sFlt-1 vs. untreated control patients at 1 year after treatment.

[00144] **FIG. 23** depicts a table of human subjects who received Lucentis rescue injections (VEGF inhibitor readministration) by week in a clinical study of rAAV.sFlt-1.

[00145] **FIG. 24** depicts visual acuity and SD-OCT images by week for a human subject treated with rAAV.sFlt-1 in a clinical study of rAAV.sFlt-1.

[00146] **FIGS. 25A** and **25B** depicts data on production of human sFlt-1 protein in human embryonic kidney 293 (HEK293) cells as detected by ELISA. rAAV.sFlt-1 was produced using plasmid transfection in HEK293 cells. A second construct, rAAV(bv).sFlt-1, was produced using recombinant baculovirus in Sf9 insect cells. sFlt-1 protein concentration was measured via ELISA after 72 at various MOI.

DETAILED DESCRIPTION OF THE DISCLOSURE

[00147] The present disclosure provides compositions and methods for the prevention or treatment of ocular neovascularization, such as AMD, in a human subject, by administering subretinally a pharmaceutical composition comprising a pharmaceutically effective amount of a vector comprising a nucleic acid encoding soluble Fms-related tyrosine kinase-1 (sFlt-1) protein to the human subject.

[00148] Several aspects of the disclosure 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 disclosure. One having ordinary skill in the relevant art, however, will readily recognize that the disclosure can be practiced without one or more of the specific details or with other methods. The present disclosure is not limited by the illustrated ordering of acts or events, as some acts may 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 present disclosure.

[00149] The terminology of the present disclosure is for the purpose of describing particular cases only and is not intended to be limiting of compositions, methods and compositions of this disclosure.

[00150] The compositions and methods of this disclosure as described herein may employ, unless otherwise indicated, conventional techniques and descriptions of molecular biology (including recombinant techniques), cell biology, biochemistry, immunochemistry and ophthalmic techniques, which are within the skill of those who practice in the art. Such conventional techniques include methods for observing and analyzing the retina, or vision in a subject, cloning and propagation of recombinant virus,

formulation of a pharmaceutical composition, and biochemical purification and immunochemistry. Specific illustrations of suitable techniques can be had by reference to the examples herein. However, equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as Green, et al., Eds., Genome Analysis: A Laboratory Manual Series (Vols. I-IV) (1999); Weiner, et al., Eds., Genetic Variation: A Laboratory Manual (2007); Dieffenbach, Dveksler, Eds., PCR Primer: A Laboratory Manual (2003); Bowtell and Sambrook, DNA Microarrays: A Molecular Cloning Manual (2003); Mount, Bioinformatics: Sequence and Genome Analysis (2004); Sambrook and Russell, Condensed Protocols from Molecular Cloning: A Laboratory Manual (2006); and Sambrook and Russell, Molecular Cloning: A Laboratory Manual (2002) (all from Cold Spring Harbor Laboratory Press); Stryer, L., Biochemistry (4th Ed.) W.H. Freeman, N.Y. (1995); Gait, "Oligonucleotide Synthesis: A Practical Approach" IRL Press, London (1984); Nelson and Cox, Lehninger, Principles of Biochemistry, 3rd Ed., W.H. Freeman Pub., New York (2000); and Berg et al., Biochemistry, 5th Ed., W.H. Freeman Pub., New York (2002), all of which are herein incorporated by reference in their entirety for all purposes. Before the present compositions, research tools and methods are described, it is to be understood that this disclosure is not limited to the specific methods, compositions, targets and uses described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to limit the scope of the present disclosure, which will be limited only by appended claims.

[00151] 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".

[00152] Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another case includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another case. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and

independently of the other endpoint. The term "about" as used herein refers to a range that is 15% plus or minus from a stated numerical value within the context of the particular usage. For example, about 10 would include a range from 8.5 to 11.5. The term "about" also accounts for typical error or imprecision in measurement of values.

I. AMD

[00153] AMD is the leading cause of blindness in patients over the age of 50 and it is characterized by progressive degeneration of the photoreceptors, outer retina, and retinal pigment epithelium at the macula. The advanced "wet" form (neovascular or exudative) of AMD is less common, but 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.

[00154] The term "AMD" if not otherwise specified, can be either dry AMD or wet AMD. The present disclosure contemplates treatment or prevention of AMD, wet AMD and/or dry AMD.

[00155] As is previously known in the art, AMD has been shown to have no single cause. This highly complex disease may result from variable contributions including but not limited to age, genetic predisposition, and environment or combination thereof. In humans, for example, established epidemiologic risk factors may include but are not limited to cigarette smoking, diet, female sex, Caucasian race, and a family history of AMD. Because AMD is rare in individuals younger than 50 years, the only required risk factor is age, which implicates the multitude of cellular changes that accompany normal aging in the pathogenesis of AMD.

[00156] The etiologic complexity of AMD is reflected by the relative paucity of effective therapies, preventive strategies, and good animal models with which to study it. Due to the complexity and incomplete characterization of the disease, AMD is incompletely modeled in animals. This is in part due to anatomical differences in animal and primate retinas, as well as the protracted time needed for the disease to develop. Evidence from human molecular genetic and animal studies support the notion that altered homeostasis of a multitude of mechanisms responsible for normal photoreceptor–RPE physiology can precipitate the disease. At least on the molecular level, the disease can be explored in

animal models and, in some cases, even in those whose gene defects are not the primary causes of AMD in humans.

[00157] Previous genetic studies as well as in depth pathological analysis, reveals that no simple inheritance pattern for AMD, and no one pathology is common to various AMD animal models. While nonhuman primate models are known in the art to better approximate CNV in humans, than mice or rat models, fundamental differences in retinal anatomy, histology and even genetics of nonhuman primates yield different species specific pathologies.

[00158] Further, and as describe herein, laser photocoagulation may be used to induce CNV, one AMD like symptom in animal models. In some cases, laser treatment ruptures the Bruch's membrane and evokes a fibrovascular proliferative response that originates in the choroid. This response is the basis for modeling choroidal neovascularization in late-stage AMD and was developed in rhesus and cynomolgus macaques.

[00159] Using an argon laser, spots are kept small and induced with sufficient power to rupture the Bruch's membrane. This is funduscopically visible as a bubble at the time of photocoagulation. Photocoagulation induces thrombosis of choroidal vessels followed by re-endothelialization 48 hours later and growth of new vessels into the subretinal space by a week. Because newly formed vessels are more permeable, neovascular development can be monitored with fluorescein angiography to assess vessel leakage.

[00160] Spontaneous neovascular involution (indicated by decreased fluorescein leakage) commences at approximately 3 to 7 weeks and then gradually progresses (over a period of approximately 2 to 13 months) until leakage is no longer apparent at the site.

[00161] The extent of new vessel growth compared to poorly vascularized scarring can be variable in all models and is influenced by species, location of injury in the retina, and intensity of the laser beam. The inherent variability in differences of treatment from species to species further supports the idea that no one animal model fully recapitulates AMD in humans.

[00162] Therapies for AMD have changed during the past few years, with the availability of aptamers, antibodies, and soluble receptor decoys that bind the protein VEGF. The VEGF protein or VEGF ligand, has been shown to stimulate the formation of new blood vessels (i.e. angiogenesis) through binding to cellular receptors, including the VEGF receptor. As known in the art, anti-VEGF agents may prevent, to some extent, the neovascularization and angiogenesis that occurs in wet AMD. Intraocular injection of

Macugen® or Lucentis® or Eylea® (anti-VEGF agents) is costly, and in most cases the treatment must be repeated every four to six weeks or every eight weeks in the case of Eylea®. For example, Lucentis is a VEGF antibody fragment which costs about $1950/inj. Monthly. Avastin (VEGF Antibody) is used off label, and Eylea (VEGF trap) costs about, $1850/inj and is administered every second month. All of these medicines share common problems of decreasing pharmacokinetic profile and thus require repeat ocular injections.

[00163] There is a need in the art for a practical, economically viable, longer lasting treatment strategy. The disclosure provides for a novel therapeutic to address some of these needs.

[00164] The present disclosure provides an anti-VEGF molecule, such as sFLT-1, delivered by any suitable vector, (e.g. recombinant viral system) to the retina of a human subject having or suspected of having AMD or related neovascular retinal diseases. In some cases, sFLT-1 may be potent direct binding protein of VEGF. In some cases, sFLT-1 may also block or inhibit VEGF activity.

[00165] For example, as known in the art, sFLT-1 (as described further herein) has been observed to bind to the VEGF protein dimer with a Kd=10 pM.

[00166] The present invention also provides compositions and methods related to rAAV mediated gene delivery into the eye. Long term gene expression in dog eyes (>8 years) has been observed with AAV based system. sFLT-1 mRNA expression in the retina is maintained at least for 18 months. Three human trials for Leber's congenital amarousis have been conducted that demonstrated the safety of an AAV based delivery system in the context of a retinal degenerative disease such as LCA.

II. VEGF and Fms-related tyrosine kinase-1 (sFLT-1) protein

A. VEGF

[00167] Vascular endothelial growth factor (herein referred to as "VEGF" or "VEGF ligand") is a potent endothelial cell-specific mitogen that plays a key role in physiological blood vessel formation. In some cases, VEGF activity results from the binding of VEGF ligand to one or more VEGF receptors in a cell. The binding of VEGF ligand to VEGF receptor may have numerous downstream cellular and biochemical effects, including but not limited to angiogenesis in tissues. VEGF has been implicated in virtually every type of angiogenic or neovascular disorder, including those associated with cancer, ischemia, and inflammation. Additionally, VEGF has been implicated in eye diseases, including

but not limited to ischemic retinopathy, intraocular neovascularization, age-related macular degeneration (AMD), wet-AMD, dry-AMD, retinal neovascularization, diabetic macular edema, diabetic retina ischemia, diabetic retinal edema, proliferative diabetic retinopathy, retinal vein occlusion, central retinal vein occlusion, branched retinal vein occlusion. Further, anti-VEGF treatments, including the compositions and methods of this disclosure as described herein, may be used in the treatment of one or more of these diseases described herein.

[00168] Recent data suggests that VEGF is the principal angiogenic growth factor in the pathogenesis of the wet form of AMD.

[00169] VEGF, a 46-kDa homodimeric glycopeptide, is expressed by several different ocular cell types including but not limited to pigment epithelial cells, pericytes, vascular endothelial cells, neuroglia and ganglion cells., In some cases, VEGF is express in specific spatial and temporal patterns during retinal development. In some cases, the human isoforms of VEGF may include proteins of 206, 189, 183, 165, 148, 145, and 121 amino acids per monomer, however the predominant human VEGF isoforms include but are not limited to VEGF121, VEGF165, VEGF189 and VEGF206. These proteins are produced by alternative splicing of the VEGF mRNA and differ in their ability to bind to heparin and to the specific VEGF receptors or coreceptors (neuropilins). The domain encoded by exons 1–5 of the VEGF gene contains information required for the recognition of the known VEGF receptors KDR/FLK-1 and FLT-1. This domain is present in all of the VEGF isoforms. VEGF acts via these receptors, which are high-affinity receptor tyrosine kinases, leading to endothelial cell proliferation, migration, and increased vasopermeability.

[00170] VEGF is one of the several factors involved in the complex process of angiogenesis and has a very high specificity for vascular endothelial cells. VEGF is a regulator of physiological angiogenesis during processes such as embryogenesis, skeletal growth and reproductive function, but it has also been implicated in pathological angiogenesis associated with disease such as in cancer, placental disorders and other conditions. The potential biological effects of VEGF may be mediated by specific fms-like membrane spanning receptors, FLT-1 and FLK-1/KDR. In some cases, these naturally occurring binding partners of VEGF may affect binding of VEGF to VEGF receptors, thus modulating activation of the VEGF receptor and subsequent downstream pathways.

[00171] As related to cancer, several VEGF inhibitors, including a humanized monoclonal antibody to VEGF (rhuMab VEGF), an anti-VEGFR-2 antibody, small molecules inhibiting VEGFR-2 signal transduction and a soluble VEGF receptor have shown some therapeutic properties.

[00172] As related to intraocular neovascular diseases, such as diabetic retinopathy, retinal vein occlusions, or age related macular degeneration, some VEGF antagonists have shown therapeutic effects, despite the need for frequent administration.

B. Anti-VEGF

[00173] The recombinant virus of the present disclosure comprises the sequence encoding an anti-VEGF protein, including, but not limited to the VEGF-binding proteins or functional fragments thereof disclosed in U.S. Pat. Nos 5,712,380, 5,861,484 and 7,071,159 and VEGF-binding fusion proteins disclosed in U.S. Pat. No. 7,635,474. An anti-VEGF protein may also include the sFLT-1 protein as described herein.

[00174] The recombinant viruses or plasmids of the present disclosure may comprise the sequence encoding an anti-VEGF protein, including the naturally occurring protein sFlt-1, as described in US Patent 5,861,484 and that sequence described by SEQ ID NO: 109. It also includes, but is not limited to functional fragments thereof, including sequences of sFlt-1 domain 2 or those set forth in SEQ ID NO: 121, as well as related constructs, such as the VEGF-binding fusion proteins disclosed in U.S. Pat. No. 7,635,474. An anti-VEGF protein may also include the sFLT-1 protein as described herein. These sequences can be expressed from DNA encoding such sequences using the genetic code, a standard technique that is understood by those skilled in the art. As can be appreciated by those with skill in the art, due to the degeneracy of the genetic code, anti-VEGF protein sequences can be readily expressed from a number of different DNA sequences.

[00175] "sFlt-1 protein" herein refers to a polypeptide sequence, or functional fragment thereof, with at least 90%, or more, homology to the naturally occurring human sFLT-1 sequence, such that the sFlt-1 protein or polypeptide binds to VEGF and/or the VEGF receptor. Homology refers to the % conservation of residues of an alignment between two sequences (e.g. a s Naturally occurring human sFLT-1 protein may include any suitable variants of sFLT-1, including, but not limited to functional fragments, sequences comprising insertions, deletions, substitutions, pseudofragments, pseudogenes, splice variants or artificially optimized sequences.In some cases, "sFLT-1 protein" may be at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99% or

100% homologous to the naturally occurring human sFLT-1 protein sequence. In some cases, "sFLT-1 protein" may be at most about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99% or 100% homologous to the naturally occurring human sFLT-1 protein sequence. In some cases, "sFLT-1 protein" may be at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99% or 100% spatially homologous to the naturally occurring human sFLT-1 protein conformation. In some cases, "sFLT-1 protein" may be at most about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99% or 100% spatially homologous to the naturally occurring human sFLT-1 protein conformation.

[00176] Further, the soluble truncated form of the VEGF receptor FLT-1, sFLT-1, is the only known endogenous specific inhibitor of VEGF. In nature, it is generated by alternative mRNA splicing and lacks the membrane-proximal immunoglobulin-like domain, the transmembrane spanning region and the intracellular tyrosine-kinase domain. Structurally, FLT-1 and sFLT-1 protein may both comprise multiple functional domains. In some variants, FLT and sFLT proteins commonly share 6 interlinked domain; 3 domains involved in dimerization of the protein and 3 domains involved in the binding of a ligand, such as VEGF.

[00177] sFLT-1 is a soluble truncated form of the FLT-1 and it is expressed endogenously. As described herein, "soluble" FLT-1, or sFLT-1 refers to FLT-1 that is not restricted to the cellular membrane. Unbound sFLT-1 may diffuse freely in extracellular space or solution.

[00178] sFLT-1 is the only known endogenous specific inhibitor of VEGF. This interaction is specific and can be competed away with 100-fold excess unlabeled VEGF. In some cases, the angiostatic activity of sFLT-1 may result from inhibition of VEGF by two mechanisms: i) sequestration of VEGF, to which it binds with high affinity, and ii) formation of inactive heterodimers with membrane-spanning isoforms of the VEGF receptors FLTt-1 and FLK-1/KDR. As known in the art, in vitro binding assays have indicate that sFLT-1 binds VEGF with high affinity and may also inhibit VEGF driven proliferation of human umbilical vein endothelial cells. In animal models for cancer, sFLT-1 inhibits tumor growth. In some cases, sFLT-1 may function in a substoichiometric or dominant negative manner, as excess VEGF in the extracellular space may be prevented from binding and subsequently activating the VEGF receptor. These properties of sFLT-1 have been described in Kendall and Thomas, 1993; *Proc Natl*

Acad Sci. 90: 10705-10709, which is incorporated herein by reference in its entirety. As is known in the art, functional fragments of sFLT-1 can be used in place of the full-length protein. More specifically, the VEGF binding domain (domain 2), or alternatively domain 2 of sFLT-1 plus domain 3 from sFLT1, KDR, or another family member, can be used to bind and inactivate VEGF. Such functional fragments are described in Wiesmann *et al.,* 1997; *Cell*, 91: 695-704, which is incorporated herein by reference in its entirety. The terms "sFLT-1" and "a functional fragment of sFLT-1" are equivalent and used here interchangeably.

III. Vectors and Recombinant Viruses

[00179] The compositions and methods of the disclosure provide for the delivery of a nucleic acid encoding an anti-VEGF (e.g. sFLT-1 proteins) to cells in a human subject or patient in need thereof. In some cases, delivery of the nucleic acid may be referred to as gene therapy.

[00180] The composition and methods of the disclosure provide for any suitable method for delivery of the anti-VEGF nucleic acid (e.g. sFLT-1). In some cases, delivery of the nucleic acid may be performed using any suitable "vector" (sometimes also referred to as "gene delivery" or "gene transfer vehicle). Vector, delivery vehicle, gene delivery vehicle or gene transfer vehicle, may refer to any suitable macromolecule or complex of molecules comprising a polynucleotide to be delivered to a target cell. In some cases, a target cell may be any cell to which the nucleic acid or gene is delivered. The polynucleotide to be delivered may comprise a coding sequence of interest in gene therapy, such as the sFLT-1 gene.

[00181] For example, suitable vectors may include but are not limited to, viral vectors such as adenoviruses, adeno-associated viruses (AAV), and retroviruses, liposomes, other lipid-containing complexes, and other macromolecular complexes capable of mediating delivery of a polynucleotide to a target cell.

[00182] In some cases, a vector may be an organic or inorganic molecule. In some cases, a vector may be small molecule (i.e. <5 kD), or a macromolecule (i.e. > 5kD). For example a vector may include but is not limited to inert, non-biologically active molecules such as metal particles. In some cases, a vector may be gold particles.

[00183] In some cases a vector may comprise a biologically active molecule. For example, vectors may comprise polymerized macromolecules such as dendrimers.

[00184] In some cases, a vector may comprise a recombinant viral vector that incorporates one or more nucleic acids. As described herein, nucleic acids may refer to polynucleotides. Nucleic acid and polynucleotide may be used interchangeably. In some cases nucleic acids may comprise DNA or RNA. In some cases, nucleic acids may include DNA or RNA for the expression of sFLT-1. In some cases RNA nucleic acids may include but are not limited to a transcript of a gene of interest (e.g. sFLT-1), introns, untranslated regions, termination sequences and the like. In other cases, DNA nucleic acids may include but are not limited to sequences such as hybrid promoter gene sequences, strong constitutive promoter sequences, the gene of interest (e.g. sFLT-1), untranslated regions, termination sequences and the like. In some cases, a combination of DNA and RNA may be used.

[00185] As described in the disclosure herein, the term "expression construct" is meant to include any type of genetic construct containing a nucleic acid or polynucleotide coding for gene products in which part or all of the nucleic acid encoding sequence is capable of being transcribed. The transcript may be translated into a protein. In some cases it may be 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.

[00186] In one aspect, the present disclosure provides a recombinant virus, such as adeno-associated virus (rAAV) as a vector to mediate the expression of sFLT-1.

[00187] In some cases, the viral vector of the disclosure may be measured as pfu (plaque forming units). In some cases, the pfu of recombinant virus, or viral vector of the compositions and methods of the disclosure may be about 10^8 to about 5 x 10^{10} pfu. In some cases, recombinant viruses of this disclosure are at least about $1x10^8$, $2x10^8$, $3x10^8$, $4x10^8$, $5x10^8$, $6x10^8$, $7x10^8$, $8x10^8$, $9x10^8$, $1x10^9$, $2x10^9$, $3x10^9$, $4x10^9$, $5x10^9$, $6x10^9$, $7x10^9$, $8x10^9$, $9x10^9$, $1x10^{10}$, $2x10^{10}$, $3x10^{10}$, $4x10^{10}$, and $5x10^{10}$ pfu. In some cases, recombinant viruses of this disclosure are at most about $1x10^8$, $2x10^8$, $3x10^8$, $4x10^8$, $5x10^8$, $6x10^8$, $7x10^8$, $8x10^8$, $9x10^8$, $1x10^9$, $2x10^9$, $3x10^9$, $4x10^9$, $5x10^9$, $6x10^9$, $7x10^9$, $8x10^9$, $9x10^9$, $1x10^{10}$, $2x10^{10}$, $3x10^{10}$, $4x10^{10}$, and $5x10^{10}$ pfu.

[00188] In some cases, the viral vector of the disclosure may be measured as vector genomes. In some cases, recombinant viruses of this disclosure are $1x10^{10}$ to $3x10^{12}$ vector genomes. In some cases, recombinant viruses of this disclosure are $1x10^9$ to $3x10^{13}$ vector genomes. In some cases, recombinant viruses of this disclosure are $1x10^8$

to $3x10^{14}$ vector genomes. In some cases, recombinant viruses of the disclosure are at least about $1x10^{1}$, $1x10^{2}$, $1x10^{3}$, $1x10^{4}$, $1x10^{5}$, $1x10^{6}$, $1x10^{7}$, $1x10^{8}$, $1x10^{9}$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, $1x10^{15}$, $1x10^{16}$, $1x10^{17}$, and $1x10^{18}$ vector genomes. In some cases, recombinant viruses of this disclosure are $1x10^{8}$ to $3x10^{14}$ vector genomes. In some cases, recombinant viruses of the disclosure are at most about $1x10^{1}$, $1x10^{2}$, $1x10^{3}$, $1x10^{4}$, $1x10^{5}$, $1x10^{6}$, $1x10^{7}$, $1x10^{8}$, $1x10^{9}$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, $1x10^{15}$, $1x10^{16}$, $1x10^{17}$, and $1x10^{18}$ vector genomes.

[00189] In some cases, the viral vector of the disclosure may be measured using multiplicity of infection (MOI). In some cases, MOI may refer to the ratio, or multiple of vector or viral genomes to the cells to which the nucleic may be delivered. In some cases, the MOI may be $1x10^{6}$. In some cases, the MOI may be $1x10^{5}$ -$1x10^{7}$. In some cases, the MOI may be $1x10^{4}$ -$1x10^{8}$. In some cases, recombinant viruses of the disclosure are at least about $1x10^{1}$, $1x10^{2}$, $1x10^{3}$, $1x10^{4}$, $1x10^{5}$, $1x10^{6}$, $1x10^{7}$, $1x10^{8}$, $1x10^{9}$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, $1x10^{15}$, $1x10^{16}$, $1x10^{17}$, and $1x10^{18}$ MOI. In some cases, recombinant viruses of this disclosure are $1x10^{8}$ to $3x10^{14}$ MOI. In some cases, recombinant viruses of the disclosure are at most about $1x10^{1}$, $1x10^{2}$, $1x10^{3}$, $1x10^{4}$, $1x10^{5}$, $1x10^{6}$, $1x10^{7}$, $1x10^{8}$, $1x10^{9}$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, $1x10^{15}$, $1x10^{16}$, $1x10^{17}$, and $1x10^{18}$ MOI.

[00190] In some aspects the nucleic acid may be delivered without the use of a virus (i.e. with a non-viral vector), and may be measured as the quantity of nucleic acid. Generally, any suitable amount of nucleic acid may be used with the compositions and methods of this disclosure. In some cases, nucleic acid may be at least about 1 pg, 10 pg, 100 pg, 1 pg, 10 pg, 100 pg, 200 pg, 300 pg, 400 pg, 500 pg, 600 pg, 700 pg, 800 pg, 900 pg, 1 μg, 10 μg, 100 μg, 200 μg, 300 μg, 400 μg, 500 μg, 600 μg, 700 μg, 800 μg, 900 μg, 1 ng, 10 ng, 100 ng, 200 ng, 300 ng, 400 ng, 500 ng, 600 ng, 700 ng, 800 ng, 900 ng, 1 mg, 10 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg 1 g, 2 g, 3 g, 4 g, or 5g. In some cases, nucleic acid may be at most about 1 pg, 10 pg, 100 pg, 1 pg, 10 pg, 100 pg, 200 pg, 300 pg, 400 pg, 500 pg, 600 pg, 700 pg, 800 pg, 900 pg, 1 μg, 10 μg, 100 μg, 200 μg, 300 μg, 400 μg, 500 μg, 600 μg, 700 μg, 800 μg, 900 μg, 1 ng, 10 ng, 100 ng, 200 ng, 300 ng, 400 ng, 500 ng, 600 ng, 700 ng, 800 ng, 900 ng, 1 mg, 10 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1 g, 2 g, 3 g, 4 g, or 5g.

[00191] In some aspects, a self-complementary vector (sc) may be used. The use of self-complementary AAV vectors may bypass the requirement for viral second-strand DNA synthesis and may lead to greater rate of expression of the transgene protein, as provided by Wu, Hum Gene Ther. 2007, 18(2):171-82, incorporated by reference herein.

[00192] In some aspects, several AAV vectors may be generated to enable selection of the most optimal serotype, promoter, and transgene.

[00193] In some cases, the vector can be a targeted vector, especially a targeted vector that selectively binds to a specific cell, such as cancer cells or tumor cells or eye cells. Viral vectors for use in the disclosure can include those that exhibit low toxicity to a target cell and induce production of therapeutically useful quantities of the anti-VEGF protein in a cell specific manner.

[00194] The compositions and methods of the disclosure provide for any suitable viral nucleic acid delivery systems including but not limited to use of at least one of an 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. Preferably, the viral vector comprises a strong eukaryotic promoter operably linked to the polynucleotide *e.g.*, a cytomegalovirus (CMV) promoter.

[00195] Generally, any suitable viral vectors may be engineered to be optimized for use with the compositions and methods of the disclosure. For example, viral vectors derived from adenovirus (Ad) or adeno-associated virus (AAV) may be used. Both human and non-human viral vectors can be used and the recombinant viral vector can be altered such that it may be replication-defective in humans. Where the vector is an adenovirus, the vector can comprise a polynucleotide having a promoter operably linked to a gene encoding the anti-VEGF protein and is replication-defective in humans.

[00196] To combine advantageous properties of two viral vector systems, hybrid viral vectors may be used to deliver a nucleic acid encoding a sFLT-1 protein to a target cell or tissue. Standard techniques for the construction of hybrid vectors are well-known to those skilled in the art. Such techniques can be found, for example, in Sambrook, et al., In Molecular Cloning: A laboratory manual. Cold Spring Harbor, N.Y. or any number of laboratory manuals that discuss recombinant DNA technology. Double-stranded AAV genomes in adenoviral capsids containing a combination of AAV and adenoviral ITRs may be used to transduce cells. In another variation, an AAV vector may be placed into a

"gutless", "helper-dependent" or "high-capacity" adenoviral vector. Adenovirus/AAV hybrid vectors are discussed in Lieber et al., J. Virol. 73:9314-9324, 1999. Retrovirus/adenovirus hybrid vectors are discussed in Zheng et al., Nature Biotechnol. 18:176-186, 2000.

[00197] Retroviral genomes contained within an adenovirus may integrate within the target cell genome and effect stable gene expression.

[00198] Replication-defective recombinant adenoviral vectors can be produced in accordance with known techniques. See, Quantin, *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:2581-2584 (1992); Stratford-Perricadet, *et al.*, *J. Clin. Invest.*, 90:626-630 (1992); and Rosenfeld, *et al.*, *Cell*, 68:143-155 (1992).

[00199] Additionally preferred vectors may include but are not limited to viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include Moloney murine leukemia viruses and HIV-based viruses. In some cases a HIV-based viral vector may be used, wherein the HIV-based viral vector comprises at least two vectors wherein the gag and pol genes are from an HIV genome and the *env* gene is from another virus. DNA viral vectors may be used. These vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as a herpes simplex I virus (HSV) vector [Geller, A.I. *et al.*, *J. Neurochem*, 64: 487 (1995); Lim, F., *et al.*, in DNA Cloning: Mammalian Systems, D. Glover, Ed. (Oxford Univ. Press, Oxford England) (1995); Geller, A.I. *et al.*, *Proc Natl. Acad. Sci.*: U.S.A.:90 7603 (1993); Geller, A.I., *et al.*, Proc Natl. Acad. Sci USA: 87:1149 (1990)], Adenovirus Vectors [LeGal LaSalle *et al.*, *Science*, 259:988 (1993); Davidson, *et al.*, *Nat. Genet.* 3: 219 (1993); Yang, *et al.*, *J. Virol.* 69: 2004 (1995)] and Adeno-associated Virus Vectors [Kaplitt, M.G., *et al.*, *Nat. Genet.* 8:148 (1994)], incorporated by reference herein.

[00200] Other viral vectors that can be used in accordance with the present disclosure include herpes simplex virus (HSV)-based vectors. HSV vectors deleted of one or more immediate early genes (IE) are advantageous because they are generally non-cytotoxic, persist in a state similar to latency in the target cell, and afford efficient target cell transduction. Recombinant HSV vectors can incorporate approximately 30 kb of heterologous nucleic acid.

[00201] Retroviruses, such as C-type retroviruses and lentiviruses, may also be used in the disclosure. For example, retroviral vectors may be based on murine leukemia virus (MLV)., as provided by Hu and Pathak, Pharmacol. Rev. 52:493511, 2000 and Fong et

al., Crit. Rev. Ther. Drug Carrier Syst. 17:1-60, 2000, incorporated by reference herein. MLV-based vectors may contain up to 8 kb of heterologous (therapeutic) DNA in place of the viral genes. The heterologous DNA may include a tissue-specific promoter and a anti-VEGF protein nucleic acid. In methods of delivery to neoplastic cells, it may also encode a ligand to a tissue specific receptor.

[00202] Additional retroviral vectors may be used including but not limited to replication-defective lentivirus-based vectors, including human immunodeficiency (HIV)-based vectors, as provided by Vigna and Naldini, J. Gene Med. 5:308-316, 2000 and Miyoshi et al., J. Virol. 72:8150-8157, 1998, incorporated by reference herein. Lentiviral vectors may be advantageous in that they are capable of infecting both actively dividing and non-dividing cells. They may also be highly efficient at transducing human epithelial cells.

[00203] Lentiviral vectors for use in the disclosure may be derived from human and non-human (including SIV) lentiviruses. Examples of lentiviral vectors include nucleic acid sequences required for vector propagation as well as a tissue-specific promoter operably linked to an anti-VEGF protein gene. Nucleic acid sequences may include the viral LTRs, a primer binding site, a polypurine tract, att sites, and an encapsidation site.

[00204] A lentiviral vector may be packaged into any suitable lentiviral capsid. The substitution of one particle protein with another from a different virus is referred to as "pseudotyping". The vector capsid may contain viral envelope proteins from other viruses, including murine leukemia virus (MLV) or vesicular stomatitis virus (VSV). The use of the VSV G-protein yields a high vector titer and results in greater stability of the vector virus particles.

[00205] Alphavirus-based vectors, such as those made from semliki forest virus (SFV) and sindbis virus (SIN), may also be used in the disclosure. Use of alphaviruses is described in Lundstrom, K., Intervirology 43:247-257, 2000 and Perri et al., Journal of Virology 74:9802-9807, 2000, incorporated by reference herein.

[00206] Recombinant, replication-defective alphavirus vectors may be advantageous because they are capable of high-level heterologous (therapeutic) gene expression, and can infect a wide target cell range. Alphavirus replicons may be targeted to specific cell types by displaying on their virion surface a functional heterologous ligand or binding domain that would allow selective binding to target cells expressing a cognate binding partner. Alphavirus replicons may establish latency, and therefore long-term heterologous

nucleic acid expression in a target cell. The replicons may also exhibit transient heterologous nucleic acid expression in the target cell.

[00207] Pox viral vectors may introduce a gene into the cell's cytoplasm. Avipox virus vectors may result in only a short term expression of the gene or nucleic acid. Adenovirus vectors, adeno-associated virus vectors and herpes simplex virus (HSV) vectors may be used with the compositions and methods of the disclosure . The adenovirus vector may result in a shorter term expression (*e.g.*, less than about a month) than adeno-associated virus, in some aspects, and may exhibit much longer expression. The particular vector chosen may depend upon the target cell and the condition being treated.

[00208] Adeno-associated viruses (AAV) are small non-enveloped single-stranded DNA viruses. They are non-pathogenic human parvoviruses and may be dependent on helper viruses, including adenovirus, herpes simplex virus, vaccinia virus and CMV, for replication. Exposure to wild-type (wt) AAV is not associated or known to cause any human pathologies and is common in the general population, usually occurring in the first decade of life in association with an adenoviral infection.

[00209] As described herein, "AAV" refers to Adeno-associated virus "rAAV" refers to a recombinant adeno-associated virus.

[00210] In some cases, the wild-type AAV encodes rep and cap genes. The rep gene is required for viral replication and the cap gene is required for synthesis of capsid proteins. Through a combination of alternative translation start and splicing sites, the small genome may be able to express four rep and three cap gene products. The rep gene products and sequences in the inverted terminal repeats (145 bp ITRs, which flank the genome) may be critical in this process. To date, 11 serotypes of AAV have been isolated. AAV2 may be used with composition and methods of the disclosure. The compositions and methods of the disclosure provide for use of any suitable AAV serotype. In some aspects, the AAV is selected from the group consisting of: AAV1, AAV2, AAV2.5, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, rh10, and hybrids thereof.

[00211] In some aspects, the present disclosure provides a recombinant virus comprising a nucleic acid further comprising a human form of the truncated, soluble VEGF receptor 1 (sFLT-1) and is named rAAV.sFlt-1. The vector is a recombinant, replicative-deficient adeno-associated viral (rAAV) vector, of serotype 2. In another aspect, the vector is a

recombinant, replicative-deficient adeno-associated viral (rAAV) vector, of serotype 2 named rAAV.sFlt-1.

[00212] AAV2 is the most characterized. rAAV2 has been shown to be able to mediate long-term transgene expression in the eyes of many species of animals. In rats, rAAV mediated reporter gene (green fluorescent protein) was still present at 18 months post injection. In monkeys, the same reported gene was present at 17 months post injection. Similarly, high sFLT-1 protein levels were present in the vitreous of rAAV.sFlt-1 injected monkey eyes at 15 months post injection.

[00213] rAAV.sFlt-1 has been tested in animal models for intraocular neovascular disorders. rAAV.sFlt-1 appeared to slow the progression of neovascularization in animal models of corneal neovascularization and retinal neovascularization. Interestingly rAAV-mediated sFlt-1 indicated some inhibition of neovascularization in a monkey model of choroidal neovascularization (model for the wet form of age related macular degeneration or AMD). In this study, the presence of the rAAV.sFlt-1 construct showed low levels of expression of sFLT-1 in the eyes of monkeys and, did not affect the well-being or retinal function of the monkeys. There is no evidence to suggest any safety issues associated with systemic exposure to rAAV.sFlt-1. The overall positive findings and lack of toxicity of rAAV vectors in these studies, as well as the findings with rAAV.sFlt-1 in mammalian models of choroidal neovascularization / AMD provide extensive supporting data that the vector has a favorable safety profile when administered to the eye.

[00214] Despite the ability of rAAV.sFlt-1 to ameleriorate certain symptoms of AMD in the monkey model, sFLT-1 proteins levels are unexpectedly low in the retina. Expression levels of sFLT-1 driven by a constitutively active mammalian promoter have been shown in the art to provide high levels of protein expression in numerous cell types. While not being bound to theory, multiple possibilities may exist for this lower than expected expression level. As a large multi-domain protein, sFLT-1 may be susceptible to premature proteolyitic degradation, poor kinetics of expression, or non optimal sorting. With respect to the latter, as a secreted protein, sFLT-1, as expressed recombinantly in cell, enters the secretory pathway. In retinal cells, including RPE cells, sFLT-1 may be secreted either apically or basolaterally, depending on either ER or Golgi appartis sorting of the protein. In some cases, non-optimal sorting may secrete the molecule to the undesired basolatteral membrane, thus decreasing the concentration of sFLT-1 molecules

available to inhibit VEGF signaling and neovascular angiogenesis on the apical surface of the RPE cell layer.

[00215] Additionally, it was unknown in the art how this unexpectedly lower level of sFLT-1 may affect efficacy of the drug towards treatment of the actual AMD disease in humans. While barely elevated levels in the monkey model showed promising signs of ameliorating symptoms of AMD, the monkey animal model for AMD merely serves a surrogate for AMD disease. As described herein, AMD symptoms are artificially induced (via laser) in the retina. While this model is suitable for various analysies, the actual efficacy of the drug in the treatment of symptoms in the monkey model is difficult to extrapolate to treatment of disease in humans. Unexpectedly lower protein levels as generated by the rAAV.sFlt-1further increases difficulty in this assessment without experiments in humans.

[00216] In addition, 3 clinical trials on Lebers Congenital Amaurosis (LCA) are being conducted in the UK and USA using the rAAV2 backbone. LCA is a rare inherited eye disease that appears at birth or in the first few months of life and it is characterized by nystagmus, sluggish or no pupillary responses, and severe vision loss or blindness. To date, no safety issues have been reported following injection of the rAAV2 construct into the subretinal space of 6 participants in these two trials. Both teams involved in the clinical trials concluded that their findings have supported further gene therapy studies In LCA patients.

[00217] Given the apparent technical difficulties in generating substantially or sustained elevated levels of sFLT-1 in monkeys, various optimization strategies may be taken to address one or more of the technical issues underlying lower protein levels of sFlt-1 in the retina after introduction of rAAV.sFlt-1. In some cases, optimization strategies, including ones as provided by the composition and methods of this disclosure may include increasing optimizing the sFlt-1protein sequence, or domains, introducing control elements to direct correct sorting after expression in retinal cells, or elevating levels of sFlt-1protein to compensate for any of these possible factors. In some cases, yhe composition and methods of the disclosure provide for specific strategies directed toward the latter, involving the incorporation of specific nucleic acid sequences directed towards improving the elevating protein levels in human retinans over sFlt-1 levels as observed previously in monkey studies. As described herein, various sequences, linkers, UTRs,

introns, sFLT-1 variants or combination thereof may be used to elevate protein levels of sFlt-1 protein in the retina after exposure to rAAV.sFlt-1.

[00218] Vectors can comprise components or functionalities that further modulate gene delivery and/or gene expression, or that otherwise provide beneficial properties to the targeted cells. Such other components include, for example, components that influence binding or targeting to cells (including components that mediate cell-type or tissue-specific binding); components that influence uptake of the vector nucleic acid by the cell; components that influence localization of the polynucleotide within the cell after uptake (such as agents mediating nuclear localization); and components that influence expression of the polynucleotide. Such components also might include markers, such as detectable and/or selectable markers that can be used to detect or select for cells that have taken up and are expressing the nucleic acid delivered by the vector. Such components can be provided as a natural feature of the vector (such as the use of certain viral vectors which have components or functionalities mediating binding and uptake), or vectors can be modified to provide such functionalities.

[00219] Selectable markers can be positive, negative or bifunctional. Positive selectable markers allow selection for cells carrying the marker, whereas negative selectable markers allow cells carrying the marker to be selectively eliminated. A variety of such marker genes have been described, including bifunctional (i.e., positive/negative) markers (see, e.g., Lupton, S., WO 92/08796, published May 29, 1992; and Lupton, S., WO 94/28143, published Dec. 8, 1994). Examples of negative selectable markers may include the inclusion of resistance genes to antibiotics, such as ampicillin or kanamycin. Such marker genes can provide an added measure of control that can be advantageous in gene therapy contexts. A large variety of such vectors are known in the art and are generally available.

[00220] In some cases, nucleic acids encoding antibiotic resistnaces markers may include but are not limited to sequences such as SEQ ID No. 110, SEQ ID No. 111, SEQ ID No. 112, SEQ ID No. 113 or SEQ ID No. 114.

[00221] In many of the viral vectors compatible with methods of the disclosure, one or more promoters can be included in the vector to allow more than one heterologous gene to be expressed by the vector. Further, the vector can comprise a sequence which encodes a signal peptide or other moiety which facilitates expression of the anti-VEGF protein from the target cell.

[00222] The nucleic acid encoding a gene product may be under transcriptional control by a promoter. A "promoter", as provided herein, refers to a suitable DNA sequence required to initiate transcription of a gene. The phrase "under transcriptional control" means that the promoter is in the correct location and orientation in relation to the nucleic acid to control RNA polymerase initiation and expression of the gene. In some cases, promoter may include a "strong" or constitutively active promoter. For example, the CMV promoter may be used as known in the art a constitutively active promoter. In some cases, the CMV promoter may comprise additional regulatory elements for promoting expression. In some cases, the CMV promoter may comprise the initial-early CMV promoter.

[00223] In some cases a promoter may refer to a "weak" promoter, or sequence that yields lower levels of sFLT-1 protein than a strong promoter. In some cases a promoter may be used such that the promoter drives selective expression of sFLT-1. In some cases a promoter or other regulatory elements used in combination with other sequences as described herein may be used to drive selective expression of sFLT-1 in an eye cell, or eye tissue.

[00224] Additionally, "promoter", **104** may also be used herein interchangeably to refer to any additional suitable transcriptional control modules that may be present around the initiation site for RNA polymerases. The compositions and methods of this disclosure may use any suitable promoters and transcriptional control modules for expression of a transgene, **106**. Additional transcriptional control modules may include but are not limited to elements such as HSV thymidine kinase (tk) and SV40 early transcription units. Generally, promoters may be composed of discrete functional modules, each consisting of approximately 7-20 bp of DNA, or 20-5000 bp of DNA, and contain one or more recognition sites for transcriptional activator or repressor proteins. The composition and methods of the disclosure provide for any suitable regulatory sequences or combination thereof. In some cases, these transcriptional control module sequences may be referred to or identified as enhancer or repressor sequences.

[00225] At least one module in each promoter functions to position the start site for RNA synthesis. One example is the TATA box. Other example may include some promoters that lack a TATA box, such as the promoter for the mammalian terminal deoxynucleotidyl transferase gene and the promoter for the SV40 late genes, a discrete element overlying the start site itself helps to fix the place of initiation.

[00226] Additional promoter elements regulate the frequency of transcriptional initiation. Generally, these are located in a region 30-110 bp upstream of the start site, although a number of promoters may contain functional elements downstream of the start site as well. The spacing between promoter elements frequently may be flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the tk promoter for example, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, individual elements may position to function either co-operatively or independently to activate transcription.

[00227] The compositions and methods of the disclosure provide for any suitable sequences for the control of expression of a nucleic acid sequence of interest in the targeted cell. Thus, where a human cell is targeted, sequences may the nucleic acid coding region may be engineered to be adjacent to and under the control of a promoter that is capable of being expressed in a human cell. Generally, such a promoter might include either a human or viral promoter.

[00228] In various aspects of the disclosure, the human cytomegalovirus (CMV) immediate early gene promoter (ie-CMV), the SV40 early promoter, the Rous sarcoma virus long terminal repeat, β-actin, rat insulin promoter and glyceraldehyde-3-phosphate dehydrogenase can be used to obtain a high level of expression of the coding sequence of interest (e.g. sFLT-1). The use of other viral or mammalian cellular or bacterial phage promoters which are well-known in the art to achieve expression of a coding sequence of interest is contemplated as well, provided that the levels of expression are sufficient for a given purpose. In some aspects, prokaryotic regulatory sequences may be present in the vector, such as the T7 RNA polymerase promoter sequence. In other aspects, the vector is free from such regulatory sequences. By employing a promoter with known properties, the level and pattern of expression of the protein of interest following transfection or transformation can be optimized.

[00229] Selection of a promoter that is regulated in response to specific physiologic or synthetic signals can permit inducible expression of the gene product. For example in the case where expression of a transgene, or transgenes when a multicistronic vector is utilized, is toxic to the cells in which the vector is produced in, it may be desirable to prohibit or reduce expression of one or more of the transgenes. Examples of transgenes that may be toxic to the producer cell line are pro-apoptotic and cytokine genes. Several

inducible promoter systems are available for production of viral vectors where the transgene product may be toxic. The composition and methods of the disclosure provide for any suitable combination of promoter sequence, regulatory sequences and transgene. In some cases, a combination of sequences may result in no toxicity to the cell. In some cases, a combination of sequences may result in high toxicity to the cell. In some cases, a combination of sequences may result in moderate levels of toxicity in the cell.

[00230] The ecdysone system (Invitrogen, Carlsbad, Calif.) is one such system for transgene expression. This system is designed to allow regulated expression of a gene of interest in mammalian cells. It consists of a tightly regulated expression mechanism that allows little basal level expression of the transgene, but over 200-fold inducibility. The system is based on the heterodimeric ecdysone receptor of Drosophila, and when ecdysone or an analog such as muristerone A binds to the receptor, the receptor activates a promoter to turn on expression of the downstream transgene high levels of mRNA transcripts are attained. In this system, both monomers of the heterodimeric receptor are constitutively expressed from one vector, whereas the ecdysone-responsive promoter which drives expression of the gene of interest is on another plasmid. Engineering of this type of system into the gene transfer vector of interest may be used in the compositions and methods of this disclosure. Cotransfection of plasmids containing the gene of interest and the receptor monomers in the producer cell line would then allow for the production of the gene transfer vector without expression of a potentially toxic transgene. At the appropriate time, expression of the transgene could be activated with ecdysone or muristeron A.

[00231] In some circumstances, it may be desirable to regulate expression of a transgene in a gene therapy vector. For example, different viral promoters with varying strengths of activity may be utilized depending on the level of expression desired. In mammalian cells, the CMV immediate early promoter may be used to provide strong transcriptional activation. Modified versions of the CMV promoter that are less potent have also been used when reduced levels of expression of the transgene are desired. When expression of a transgene in hematopoietic cells is desired, retroviral promoters such as the LTRs (Long Terminal Repeat) from MLV or MMTV are often used. Other viral promoters that may be used depending on the desired effect include SV40, RSV LTR, HIV-1 and HIV-2 LTR, adenovirus promoters such as from the E1A, E2A, or MLP region, AAV LTR, cauliflower mosaic Virus, HSV-TK, and avian sarcoma virus.

[00232] In some aspects, tissue-specific promoters are used to effect transcription in specific tissues or cells so as to reduce potential toxicity or undesirable effects to non-targeted tissues. For example, promoters such as the PSA, probasin, prostatic acid phosphatase or prostate-specific glandular kallikrein (hK2) may be used to target gene expression in the prostate.

[00233] In some cases, promoters or regulatory sequence elements may be used to direct selective expression in eye cells or eye tissue. For example, promoter, sequence elements or regulatory sequences found in specific eye cell types, such as retinal pigment epithelial cells, may be used in a suitable expression construct (e.g., the RPE65 or VMD2 promoter).

[00234] The selection of appropriate promoters can be readily accomplished. In some cases a high expression, or strong promoter may be used. An example of a suitable promoter is the 763-base-pair cytomegalovirus (CMV) promoter. The Rous sarcoma virus (RSV) (Davis, *et al.*, *Hum Gene Ther* 4:151 (1993)) and MMT promoters may also be used. Certain proteins can be expressed using their native promoter. Other elements that can enhance expression can also be included such as an enhancer or a system that results in high levels of expression such as a tat gene and tar element. This cassette can then be inserted into a vector, *e.g.*, a plasmid vector such as, pUC19, pUC118, pBR322, or other known plasmid vectors, that includes, for example, an *E. coli* origin of replication. See, Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory press, (1989). Promoters are discussed *infra*. The plasmid vector may also include a selectable marker such as the β-lactamase gene for ampicillin resistance, provided that the marker polypeptide does not adversely affect the metabolism of the organism being treated. The cassette can also be bound to a nucleic acid binding moiety in a synthetic delivery system, such as the system disclosed in WO 95/22618, incorporated by reference herein. Generally promoter sequences and/or any associated regulatory sequences may comprise about at least 150 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1000 bp, 2000 bp, 3000 bp, 4000 bp,5000 bp or 10000 bp. Promoter sequences and any associated regulatory sequences, may comprise about at most 150 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1000 bp, 2000 bp, 3000 bp, 4000 bp,5000 bp or 10000 bp.

[00235] In some aspects, the recombinant virus or plasmid comprises a promoter selected from cytomegalovirus (CMV) promoter, Rous sarcoma virus (RSV) promoter, and MMT

promoter, EF-1 alpha promoter, UB6 promoter, chicken beta-actin promoter, CAG promoter, RPE65 promoter and opsin promoter. Generally, promoter sequences and promoter/enhancer sequences as provided by the present disclosure may include but are not limited to any sequences selected from 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.

[00236] In some aspects, an antibiotic marker is used in the process for production of the recombinant virus. Antibiotic resistance markers may be used to identify positive transgenic cells in the generation of recombinant virus. In some aspects, the antibiotic marker comprises a sequence encoding an antibiotic resistance gene, such as those provided herein including but not limited to sequences shown in Fig. 8A and Fig. 8B. For example markers conferring resistance may include but are not limited to kanamycin, gentamicin, ampicillin, chloramphenicol, tetracycline, doxycycline, or hygromycin. In some aspects, the antibiotic resistance gene is a non-beta-lactam antibiotic resistance gene such as kanamycin.

[00237] In some aspects, the recombinant virus and/or plasmid used to generate recombinant virus, comprise a sequence encoding a replication origin sequence, such as those provided herein. Origin of replication sequences, generally provide sequence useful for propagating a plasmid. Generally, origin of replication sequences as provided by the present disclosure may include but are not limited to any sequences selected from sequences as provided in Fig. 7A, Fig. 7B, Fig. 7C and Fig. 7D.

[00238] In some aspects, an origin or origin of replication sequences may include but is not limited to sequences such as SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13, SEQ ID No. 14, SEQ ID No. 15, SEQ ID No. 16, or SEQ ID No. 17.

[00239] In some aspects, the recombinant virus and/or plasmid used to generate recombinant virus, comprise an enhancer, such as those provided herein.

[00240] In some aspects, the recombinant virus and/or plasmid used to generate recombinant virus, comprise a chimeric intron or an intron, **105**, such as those provided herein and disclosed in U.S. Pat. No. 7635474, incorporated by reference herein. Intron or chimeric intron may be used interchangeably herein. In some cases, an intron may refer to any sequence that may be transcribed but is not translated. In some cases, an intron may refer to any sequence that be transcribed and is removed from a mature RNA transcript in a cell. In some cases, an intron may comprise about at least 1 bp, 50 bp, 100 bp, 150 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1000 bp, 2000 bp, 3000 bp, 4000 bp or 5000 bp. In some cases, an intron may comprise may comprise about at least 1 bp, 50 bp, 100 bp, 150 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1000 bp, 2000 bp, 3000 bp, 4000 bp or 5000 bp. In some cases, an intron may be about 300 bp. In some cases, an intron may be about 200-400 bp. In some cases, a chimeric intron may be about 100 – 500 bp. In some cases, an intron may be about 50 – 200 bp. In some cases, an intron may be either an intact naturally occurring intron or a chimeric intron.

[00241] In some aspects, an intron may include but is not limited to sequences such as SEQ ID No. 48, SEQ ID No. 115, SEQ ID No. 116, SEQ ID No. 117, SEQ ID No. 118, SEQ ID No. 119 or SEQ ID No. 120.

[00242] In some aspects, the recombinant virus and/or plasmid used to generate recombinant virus, comprise a poly A (polyadenylation) sequence, **107**, such as those provided herein (e.g. SV40 poly A sequence.). Generally, any suitable polyA sequence may be used for the desired expression of the transgene (i.e. sFLT-1). For example, in some cases, the present disclosure provides for a sequence comprising SV40 polyA sequence, or portion of SV40 polyA sequence. In some cases, native polyA sequences as found downstream (3'UTR) of the human sFLT-1 gene as found in human genomic sequence may be used. In other cases, polyA sequences as found downstream of genes other than sFLT-1 may be used. In other cases, the present disclosure provides for polyA sequences comprising a combination of one or more polyA sequences or sequence elements. In some cases, no polyA sequence is used. In some cases one or more polyA sequences may be referred to as untranslated regions (UTRs), 3' UTRs, or termination sequences.

[00243] In certain aspects of the disclosure, the use of internal ribosome entry site (IRES) or foot-mouth disease virus (FMDV) elements may be used to create multigene, or

polycistronic, messages. IRES elements are able to bypass the ribosome scanning model of 5' methylated Cap dependent translation and begin translation at internal sites. IRES elements from two members of the picornavirus family (poliovirus and encephalomyocarditis) have been described, as well an IRES from a mammalian message. IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. By virtue of the IRES element, each open reading frame may be accessible to ribosomes for efficient translation. Multiple genes can be efficiently expressed using a single promoter/enhancer to transcribe a single message. An alternative system for co-expression of two proteins in gene therapy delivery vectors is the FMDV 2A system. The FMDV 2A system employs a retroviral plasmid vector in which two genes may be linked to a nucleotide sequence encoding the 2A sequence from the picornavirus foot-and-mouth disease virus. Transcription and translation gives rise to a bicistronic mRNA and two independent protein products.

[00244] Any heterologous open reading frame can be linked to IRES elements. This may include genes for secreted proteins, multi-subunit proteins, encoded by independent genes, intracellular or membrane-bound proteins and selectable markers. In this way, expression of several proteins can be simultaneously engineered into a cell with a single construct and a single selectable marker.

[00245] A polyA sequence may comprise a length of 1 - 10 bp, 10- 20 bp, 20 - 50 bp, 50 - 100 bp, 100 - 500 bp, 500 bp - 1Kb, 1Kb - 2 Kb, 2Kb - 3 Kb, 3Kb - 4 Kb, 4Kb - 5 Kb, 5Kb - 6 Kb, 6Kb -7 Kb, 7Kb - 8 Kb, 8Kb - 9 Kb, and 9Kb - 10 Kb in length. A polyA sequence may comprise a length of at least 1 bp, 2 bp, 3 bp, 4 bp, 5 bp, 6 bp, 7 bp, 8 bp, 9 bp, 10 bp, 20 bp, 30 bp, 40 bp, 50 bp, 60 bp, 70 bp, 80 bp, 90 bp, 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1Kb, 2 Kb, 3 Kb, 4 Kb, 5 Kb, 6 Kb, 7 Kb, 8 Kb, 9 Kb, and 10 Kb in length. A polyA sequence may comprise a length of at most 1 bp, 2 bp, 3 bp, 4 bp, 5 bp, 6 bp, 7 bp, 8 bp, 9 bp, 10 bp, 20 bp, 30 bp, 40 bp, 50 bp, 60 bp, 70 bp, 80 bp, 90 bp, 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1Kb, 2 Kb, 3 Kb, 4 Kb, 5 Kb, 6 Kb, 7 Kb, 8 Kb, 9 Kb, and 10 Kb in length.

[00246] In some cases, a polyA or termination sequence may include but is not limited to sequences such as 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.

[00247] Generally, polyA sequences, as provided by the present disclosure, may include but are not limited to any sequences selected from PolyA Regions 1-10 as provided in Fig. 9A and Fig. 9B.

[00248] In some cases, polyA sequences may be optimized for various parameters affecting protein expression, including but not limited to mRNA half-life of the transgene in the cell, stability of the mRNA of the transgene or transcriptional regulation. For example, polyA sequences maybe altered to increase mRNA transcript of the transgene, which may result in increased protein expression. In some cases, the polyA sequences maybe altered to decrease the half-life of the mRNA transcript of the transgene, which may result in decreased protein expression.

[00249] In some aspects, the recombinant virus and/or plasmid used to generate recombinant virus, comprise a polynucleotide encoding a human sFLT-1 protein or a functional fragment thereof. In some cases, the recombinant virus and/or plasmid used to generate recombinant virus, comprises a nucleic acid encoding another anti-VEGF protein or VEGF inhibitor.

[00250] In some cases, a VEGF inhibitor may include but is not limited to sequences such as SEQ ID No. 102, SEQ ID No. 103, SEQ ID No. 104, SEQ ID No. 105, SEQ ID No. 106, SEQ ID No. 107, SEQ ID No. 108, or SEQ ID No. 122

[00251] In some cases, nucleic acids of a VEGF inhibitor may encode for polypeptide sequences which may include but are not limited to polypeptide sequences such as SEQ ID No. 109 or SEQ ID No. 121.

[00252] In some aspects, the recombinant virus and/or plasmid used to generate recombinant virus, comprise a regulatory nucleic acid fragment that is capable of directing selective expression of the sFLT-1 protein in an eye cell. In some cases, eye cells may comprise retinal pigment epithelial cells (RPE).

[00253] In some aspects, the recombinant virus and/or plasmid used to generate recombinant virus, may comprise one or more untranslated regions (UTR) or sequences. Generally, any suitable UTR sequence may be used for the desired optimal expression of the transgene (i.e. sFLT-1). For example, in some cases, UTR regions or sequences may comprise native sequences. In some cases, UTR sequences may be sequences as found upstream (5' UTR) or downstream (3'UTR) of the human sFLT-1 gene as found in human genomic sequence or portions thereof. In other cases, UTR sequences may comprise non-native sequences, such as found upstream or downstream of genes other

than sFLT-1 or comprise sequences further comprising a combination of one or more UTR sequence elements as further described herein. In some cases, only a 5' UTR sequence is used. In some cases, only a 3' UTR sequence is used. In some cases, no UTR sequences are used.

[00254] A UTR sequence may comprise a length of 1 - 10 bp, 10- 20 bp, 20 - 50 bp, 50 - 100 bp, 100 - 500 bp, 500 bp - 1Kb, 1Kb - 2 Kb, 2Kb - 3 Kb, 3Kb - 4 Kb, 4Kb - 5 Kb, 5Kb - 6 Kb, 6Kb -7 Kb, 7Kb - 8 Kb, 8Kb - 9 Kb, and 9Kb - 10 Kb in length. A UTR sequence may comprise a length of at least 1 bp, 2 bp, 3 bp, 4 bp, 5 bp, 6 bp, 7 bp, 8 bp, 9 bp, 10 bp, 20 bp, 30 bp, 40 bp, 50 bp, 60 bp, 70 bp, 80 bp, 90 bp, 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1Kb, 2 Kb, 3 Kb, 4 Kb, 5 Kb, 6 Kb, 7 Kb, 8 Kb, 9 Kb, and 10 Kb in length. A UTR sequence may comprise a length of at most 1 bp, 2 bp, 3 bp, 4 bp, 5 bp, 6 bp, 7 bp, 8 bp, 9 bp, 10 bp, 20 bp, 30 bp, 40 bp, 50 bp, 60 bp, 70 bp, 80 bp, 90 bp, 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1Kb, 2 Kb, 3 Kb, 4 Kb, 5 Kb, 6 Kb, 7 Kb, 8 Kb, 9 Kb, and 10 Kb in length.

[00255] Generally, UTR sequences as provided by the present disclosure may include but are not limited to any sequences including but to limited to 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.

[00256] In some cases, variations of either the 5'UTR and/or 3'UTR may be optimized for a desired level of protein expression. In some cases, 3'UTR sequences may be optimized for various parameters affecting protein expression, including but not limited to mRNA half-life of the transgene in the cell, stability or secondary structure of the mRNA of the transgene or conditional regulation (e.g. binding of various factors to modulate translation). For example, the 3'UTR sequence maybe altered to increase the half-life of the mRNA transcript of the transgene, which may result in increased protein expression. In some cases, the 3'UTR sequence maybe altered to decrease the half-life of the mRNA transcript of the transgene, which may result in decreased protein expression.

[00257] Generally, 3' UTRs sequences may comprise various sequence elements. The present disclosure provides for 3' UTR sequences that may include but are not limited to sequence elements such as one or more polyadenylation signals, linker sequences, spacer sequences, SECIS elements, AU-rich or ARE sequences or miRNA or RNAi binding sequences, transcription terminator sequences, 3' termination sequences or variants and/or combinations thereof.

[00258] In some cases, 5'UTR sequences may be optimized for various parameters affecting protein expression, including but not limited to mRNA half-life of the transgene in the cell, stability or secondary structure of the mRNA of the transgene or transcriptional regulation. For example, the 5'UTR sequences maybe altered to increase translation efficiency of mRNA transcript of the transgene, which may result in increased protein expression. In some cases, the 5'UTR sequences maybe altered to decrease translation efficiency of mRNA transcript of the transgene, which may result in decreased protein expression.

[00259] Generally, 5' UTRs sequences may comprise various sequence elements. The present disclosure provides for 5' UTR sequences that may include but are not limited to sequence elements such as one or more ribosome binding sites (RBS), linker sequences, spacer sequences, regulatory sequences, regulatory response elements, riboswitches, sequences that promote or inhibit translation initiation, regulatory sequences for mRNA transport or variants and/or combinations thereof.

[00260] In some aspects, the recombinant virus and/or plasmid used to generate recombinant virus, may comprise one or more linker or spacer sequences. As described herein, linker sequence or spacer sequence may be used interchangeably. Generally, a linker sequence or spacer sequence may be any suitable sequence used to create a non-contiguous sequence between at least two sequence elements. For example, in one aspect of the disclosure, a linker sequence may be found inserted between an ITR-1, **108** sequence, or ITR-2, **103**, and an antibiotic resistance gene sequence, **106** as reflected in Fig. 1A. In another example, linker sequences may be inserted adjacent to any sequence element of the recombinant virus or the plasmid encoding the recombinant virus including the ITR sequences, the promoter or promoter/enhancer sequences, the intron sequence, the transgene sequence and the poly A region sequence. Generally, any suitable linker or spacer sequence may be used to create non-contiguous sequences. For example, in some cases, linker sequences may be randomly generated sequence. In some cases, linker sequence may be non-specific sequence optimized to prevent formation of secondary structure or intramolecular interactions that may adversely affect protein expression. In some cases, linker sequences may comprise any additional functional sequence elements, including but not limited to introns, regulatory sequences, enhancers or the like. Functional elements in linker sequences may be used for the desired optimal production of virus and/or expression of transgene expression. In some cases, linker sequences are

cloning sites, remnants of prior cloning sites or other non-significant sequences and the insertion of such linkers between any two sequence elements is optional.

[00261] Generally, linker sequence, as provided by the present disclosure, may include but are not limited to any sequences selected from sequences as provided in Fig. 9D, Fig. 9E and Fig. 9F.

[00262] In some cases, the length of the linker sequence may be optimized for the desired optimal production of virus and/or expression of transgene expression. In some cases, the length of one or more linker sequences located at one or more sites in the virus genome or plasmid may be varied to produce the desired optimal protein expression. For example, a linker sequence may be found between the intron, as described herein and the transgene (i.e. sFLT-1). The length of the linker sequence may be varied to produce varying effects on the transcription and subsequent translation of the transgene in the cell.

[00263] A linker sequence may comprise a length of 1 - 10 bp, 10- 20 bp, 20 - 50 bp, 50 - 100 bp, 100 - 500 bp, 500 bp - 1Kb, 1Kb - 2 Kb, 2Kb - 3 Kb, 3Kb - 4 Kb, 4Kb - 5 Kb, 5Kb - 6 Kb, 6Kb -7 Kb, 7Kb - 8 Kb, 8Kb - 9 Kb, and 9Kb - 10 Kb in length. A linker sequence may comprise a length of at least 1 bp, 2 bp, 3 bp, 4 bp, 5 bp, 6 bp, 7 bp, 8 bp, 9 bp, 10 bp, 20 bp, 30 bp, 40 bp, 50 bp, 60 bp, 70 bp, 80 bp, 90 bp, 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1Kb, 2 Kb, 3 Kb, 4 Kb, 5 Kb, 6 Kb, 7 Kb, 8 Kb, 9 Kb, and 10 Kb in length. A linker sequence may comprise a length of at most 1 bp, 2 bp, 3 bp, 4 bp, 5 bp, 6 bp, 7 bp, 8 bp, 9 bp, 10 bp, 20 bp, 30 bp, 40 bp, 50 bp, 60 bp, 70 bp, 80 bp, 90 bp, 100 bp, 200 bp, 300 bp, 400 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1Kb, 2 Kb, 3 Kb, 4 Kb, 5 Kb, 6 Kb, 7 Kb, 8 Kb, 9 Kb, and 10 Kb in length.

[00264] In some cases, a linker or spacer sequence may include but is not limited to SEQ ID No. 60, SEQ ID No. 61, SEQ ID No. 62, SEQ ID No. 63, SEQ ID No. 64, SEQ ID No. 65, SEQ ID No. 66, SEQ ID No. 67, SEQ ID No. 68, SEQ ID No. 69, SEQ ID No.70, SEQ ID No. 71, SEQ ID No. 72, SEQ ID No. 73, SEQ ID No. 74, SEQ ID No. 75, SEQ ID No. 76, SEQ ID No. 77, SEQ ID No. 78, SEQ ID No. 79, SEQ ID No. 80, SEQ ID No. 81, SEQ ID No. 82, SEQ ID No. 83, SEQ ID No. 84, SEQ ID No. 85, SEQ ID No. 86, SEQ ID No. 87, SEQ ID No. 88, SEQ ID No. 89, and SEQ ID No. 90.

[00265] In some aspects, the recombinant virus comprises inverted terminal repeat (ITR) sequences used for packaging the recombinant gene expression cassette into the virion of the viral vector. In some cases, the ITR is from adeno-associated virus (AAV). In some

cases, the ITR is from AAV serotype 2. In some cases, an ITR may include but is not limited to SEQ ID No. 56, SEQ ID No. 57, SEQ ID No. 58, or SEQ ID No. 59.

[00266] In some aspects, the recombinant virus and/or plasmid used to generate recombinant virus comprises nucleic acid elements in the following order: a) a first ITR sequence; b) a promoter sequence; c) an intron sequence; d) a first UTR sequence; e) a sequence encoding a VEGF inhibitor; f) a second UTR sequence; g) a poly A sequence; and h) a second ITR sequence. In some aspects of the recombinant virus and/or plasmid used to generate the recombinant virus, the promoter sequence comprises a promoter/enhancer sequence. In some aspects, the sequence encoding a VEGF inhibitor comprises a sequence encoding human sFLT-1 protein or a functional fragment thereof. In other aspects, the plasmid used to generate the recombinant virus further comprises an origin of replication sequence, **102**. In some aspects, the plasmid further comprises a sequence for an antibiotic resistance gene as provided herein.

[00267] In some aspects, the recombinant virus and/or plasmid used to generate recombinant virus comprises nucleic acid elements in the following order: a) a first ITR sequence; b) a first linker sequence; c) a promoter sequence; d) a second linker sequence; e) an intron sequence; f) a third linker sequence; g) a first UTR sequence; h) a sequence encoding a VEGF inhibitor; i) a second UTR sequence; j) a fourth linker sequence; k) a poly A sequence; l) a fifth linker sequence; and m) a second ITR sequence. In some aspects of the recombinant virus and/or plasmid used to generate recombinant virus, the promoter sequence comprises a promoter/enhancer sequence. In some aspects, the sequence encoding a VEGF inhibitor comprises a sequence encoding human sFLT-1 protein or a functional fragment thereof. In other aspects, the plasmid used to generate the recombinant virus further comprises an origin of replication sequence. In some aspects, the plasmid further comprises a sequence for an antibiotic resistance gene as provided herein.

IV. Pharmaceutical Compositions

[00268] A pharmaceutical composition is a formulation containing one or more active ingredients as well as one or more excipients, carriers, stabilizers or bulking agents, which is suitable for administration to a human patient to achieve a desired diagnostic result or therapeutic or prophylactic effect. For storage stability and convenience of handling, a pharmaceutical composition can be formulated as a lyophilized (i.e. freeze dried) or vacuum dried powder which can be reconstituted with saline or water prior to

administration to a patient. Alternately, the pharmaceutical composition can be formulated as an aqueous solution. A pharmaceutical composition can contain a proteinaceous active ingredient. Unfortunately, proteins can be very difficult to stabilize, resulting in loss of protein and/or loss of protein activity during the formulation, reconstitution (if required) and during the storage prior to use of a protein containing pharmaceutical composition. Stability problems can occur because of protein denaturation, degradation, dimerization, and/or polymerization. Various excipients, such as albumin and gelatin have been used with differing degrees of success to try and stabilize a protein active ingredient present in a pharmaceutical composition. Additionally, cryoprotectants such as alcohols have been used to reduce protein denaturation under the freezing conditions of lyophilization.

[00269] Pharmaceutical compositions suitable for internal use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures 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 (Tween™), 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, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars,

polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the internal compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[00270] Sterile solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof

[00271] In one aspect, active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811, incorporated by reference herein.

[00272] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the human subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[00273] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[00274] The pharmaceutical compositions of the disclosure encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal comprising a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to prodrugs and pharmaceutically acceptable salts of the compounds of the disclosure, pharmaceutically acceptable salts of such prodrugs, and other bio-equivalents.

[00275] The term "prodrug" indicates a therapeutic agent that is prepared in an inactive form that is converted to an active form (*i.e.*, drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions.

[00276] The term "pharmaceutically acceptable salt" refers to physiologically and pharmaceutically acceptable salts of the compounds of the disclosure: *i.e.*, salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

[00277] Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Metals used as cations comprise sodium, potassium, magnesium, calcium, and the like. Amines comprise N-N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge *et al.*, "Pharmaceutical Salts," *J. Pharma Sci.*, 1977, 66, 119). The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present disclosure.

[00278] As used herein, a "pharmaceutical addition salt" comprises a pharmaceutically acceptable salt of an acid form of one of the components of the compositions of the disclosure. These comprise organic or inorganic acid salts of the amines. Preferred acid salts are the hydrochlorides, acetates, salicylates, nitrates and phosphates. Other suitable pharmaceutically acceptable salts are well known to those skilled in the art and comprise

basic salts of a variety of inorganic and organic acids, such as, for example, with inorganic acids, such as for example hydrochloric acid, hydrobromic acid, sulfuric acid or phosphoric acid; with organic carboxylic, sulfonic, sulfo or phospho acids or N-substituted sulfamic acids, for example acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, fumaric acid, malic acid, tartaric acid, lactic acid, oxalic acid, gluconic acid, glucaric acid, glucuronic acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, salicylic acid, 4-aminosalicylic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, embonic acid, nicotinic acid or isonicotinic acid; and with amino acids, such as the 20 alpha-amino acids involved in the synthesis of proteins in *Nature*, for example glutamic acid or aspartic acid, and also with phenylacetic acid, methanesulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, benzenesulfonic acid, 4-methylbenzenesulfoic acid, naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic acid, 2-or 3-phosphoglycerate, glucose-6-phosphate, N-cyclohexylsulfamic acid (with the formation of cyclamates), or with other acid organic compounds, such as ascorbic acid. Pharmaceutically acceptable salts of compounds may also be prepared with a pharmaceutically acceptable cation. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and comprise alkaline, alkaline earth, ammonium and quaternary ammonium cations. Carbonates or hydrogen carbonates are also possible. For oligonucleotides, preferred examples of pharmaceutically acceptable salts comprise but are not limited to: (I) salts formed with cations such as sodium, potassium, ammonium, magnesium, calcium, polyamides such as spermine and spermidine, and the like; (II) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; (III) salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, napthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (IV) salts formed from elemental anions such as chlorine, bromine, and iodine.

[00279] Pharmaceutical compositions of the present disclosure comprise, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that comprise, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

[00280] Certain compositions of the present disclosure also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (*i.e.*, does not possess biological activity per se) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The co-administration of a nucleic acid and a carrier compound, generally with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extra circulatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate oligonucleotide in hepatic tissue can be reduced when it is co-administered with polyinosinic acid, dextran sulphate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'disulfonic acid (Miyao *et al.*, *Antisense Res. Dev.*, 1995, 5, 115-121; Takakura *et al.*, *Antisense & Nucl. Acid Drug Dev.*, 1996, 6, 177-183).

[00281] The vector or recombinant viruses (virions) can be incorporated into pharmaceutical compositions for administration to mammalian patients, particularly humans. The vector or virions can be formulated in nontoxic, inert, pharmaceutically acceptable aqueous carriers, preferably at a pH ranging from 3 to 8, more preferably ranging from 6 to 8. Such sterile compositions will comprise the vector or virion containing the nucleic acid encoding the therapeutic molecule dissolved in an aqueous buffer having an acceptable pH upon reconstitution.

[00282] In some aspects, the pharmaceutical composition provided herein comprise a therapeutically effective amount of a vector or virion in admixture with a pharmaceutically acceptable carrier and/or excipient, for example saline, phosphate buffered saline, phosphate and amino acids, polymers, polyols, sugar, buffers, preservatives and other proteins. Exemplary amino acids, polymers and sugars and the like are octylphenoxy polyethoxy ethanol compounds, polyethylene glycol monostearate compounds, polyoxyethylene sorbitan fatty acid esters, sucrose, fructose, dextrose, maltose, glucose, mannitol, dextran, sorbitol, inositol, galactitol, xylitol, lactose, trehalose, bovine or human serum albumin, citrate, acetate, Ringer's and Hank's solutions, cysteine, arginine, carnitine, alanine, glycine, lysine, valine, leucine,

polyvinylpyrrolidone, polyethylene and glycol. Preferably, this formulation is stable for at least six months at 4° C.

[00283] In some aspects, the pharmaceutical composition provided herein comprises a buffer, such as phosphate buffered saline (PBS) or sodium phosphate/sodium sulfate, tris buffer, glycine buffer, sterile water and other buffers known to the ordinarily skilled artisan such as those described by Good et al. (1966) Biochemistry 5:467. The pH of the buffer in which the pharmaceutical composition comprising the anti-VEGF contained in the adenoviral vector delivery system, may be in the range of 6.5 to 7.75, 7 to 7.5, or 7.2 to 7.4. The pH of the formulation may range from about 3.0 to about 12.0. The pH of the immunogenic composition may be at least about 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 pH units. The pH of the immunogenic composition may be at most about 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 pH units.

[00284] In some aspects, the pharmaceutical composition provided herein comprises substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran, in the amount about 1-10 percent, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 percent.

[00285] Certain aspects of the disclosure provide pharmaceutical compositions containing one or more recombinant virus and one or more other chemotherapeutic agents.

[00286] Examples of such chemotherapeutic agents comprise, but are not limited to, anticancer drugs such as daunorubicin, dactinomycin, doxorubicin, bleomycin, mitomycin, nitrogen mustard, chlorambucil, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-fluorouracil (5-FU), floxuridine (5-FUdR), methotrexate (MIX), colchicine, vincristine, vinblastine, etoposide, teniposide, cisplatin and diethylstilbestrol (DES). See, generally, The Merck Manual of Diagnosis and Therapy, 15th Ed., Berkow *et al.*, eds., 1987, Rahway, N.J., pages 1206-1228).

[00287] Anti-inflammatory drugs, comprising but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, comprising but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the disclosure (The Merck Manual of Diagnosis and Therapy, 15th Ed., Berkow *et al.*, eds., 1987, Rahway, N.J., pages 2499-2506 and 46-49, respectively). Other non-antisense chemotherapeutic agents are also within the scope of this disclosure. Two or more combined compounds may be used together or sequentially.

[00288] In another related aspect, compositions of the disclosure may contain one or more recombinant viruses, particularly sFLT-1 with different sequences. Two or more combined viruses may be used together or sequentially.

[00289] In another aspect, the present disclosure provides a unit dose of a pharmaceutical composition comprising about 1 x 10^6 about 1 x 10^{15} viral genomes, wherein the viruses comprises a nucleic acid encoding sFLT-1.

[00290] In some cases, the unit dose of the pharmaceutical composition of the disclosure may be measured as pfu (plaque forming units). In some cases, the pfu of the unit dose of the pharmaceutical composition of the disclosure may be about $1x10^8$ to about $5x10^{10}$ pfu. In some cases, the pfu of the unit dose of the pharmaceutical composition of the disclosure is at least about $1x10^8$, $2x10^8$, $3x10^8$, $4x10^8$, $5x10^8$, $6x10^8$, $7x10^8$, $8x10^8$, $9x10^8$, $1x10^9$, $2x10^9$, $3x10^9$, $4x10^9$, $5x10^9$, $6x10^9$, $7x10^9$, $8x10^9$, $9x10^9$, $1x10^{10}$, $2x10^{10}$, $3x10^{10}$, $4x10^{10}$, and $5x10^{10}$ pfu. In some cases, the pfu of the unit dose of the pharmaceutical composition of the disclosure is at most about $1x10^8$, $2x10^8$, $3x10^8$, $4x10^8$, $5x10^8$, $6x10^8$, $7x10^8$, $8x10^8$, $9x10^8$, $1x10^9$, $2x10^9$, $3x10^9$, $4x10^9$, $5x10^9$, $6x10^9$, $7x10^9$, $8x10^9$, $9x10^9$, $1x10^{10}$, $2x10^{10}$, $3x10^{10}$, $4x10^{10}$, and $5x10^{10}$ pfu.

[00291] In some cases, the viral vector of the disclosure may be measured as vector genomes. In some cases, the unit dose of the pharmaceutical composition of the disclosure is $1x10^{10}$ to $3x10^{12}$ vector genomes. In some cases, the unit dose of the pharmaceutical composition of the disclosure is $1x10^9$ to $3x10^{13}$ vector genomes. In some cases, the unit dose of the pharmaceutical composition of the disclosure is $1x10^{10}$ to $1x10^{11}$ vector genomes. In some cases, the unit dose of the pharmaceutical composition of the disclosure is $1x10^8$ to $3x10^{14}$ vector genomes. In some cases, the unit dose of the pharmaceutical composition of the disclosure is at least about $1x10^1$, $1x10^2$, $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, $1x10^{15}$, $1x10^{16}$, $1x10^{17}$, and $1x10^{18}$ vector genomes. In some cases, the unit dose of the pharmaceutical composition of the disclosure is $1x10^8$ to $3x10^{14}$ vector genomes. In some cases, the unit dose of the pharmaceutical composition of the disclosure is at most about $1x10^1$, $1x10^2$, $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, $1x10^{15}$, $1x10^{16}$, $1x10^{17}$, and $1x10^{18}$ vector genomes.

[00292] In some cases, the unit dose of the pharmaceutical composition of the disclosure may be measured using multiplicity of infection (MOI). In some cases, MOI may refer to the ratio, or multiple of vector or viral genomes to the cells to which the nucleic may be

delivered. In some cases, the MOI may be $1x10^6$. In some cases, the MOI may be $1x10^5$ -$1x10^7$. In some cases, the MOI may be $1x10^4$ -$1x10^8$. In some cases, recombinant viruses of the disclosure are at least about $1x10^1$, $1x10^2$, $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, $1x10^{15}$, $1x10^{16}$, $1x10^{17}$, and $1x10^{18}$ MOI. In some cases, recombinant viruses of this disclosure are $1x10^8$ to $3x10^{14}$ MOI. In some cases, recombinant viruses of the disclosure are at most about $1x10^1$, $1x10^2$, $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, $1x10^{15}$, $1x10^{16}$, $1x10^{17}$, and $1x10^{18}$ MOI.

[00293] In some aspects the nucleic acid may be delivered without the use of a virus (i.e. with a non-viral vector), and may be measured as the quantity of nucleic acid. Generally, any suitable amount of nucleic acid may be used with the compositions and methods of this disclosure. In some cases, the amount of nucleic acid may be at least about 1 pg, 10 pg, 100 pg, 1 pg, 10 pg, 100 pg, 200 pg, 300 pg, 400 pg, 500 pg, 600 pg, 700 pg, 800 pg, 900 pg, 1 ng, 10 ng, 100 ng, 200 ng, 300 ng, 400 ng, 500 ng, 600 ng, 700 ng, 800 ng, 900 ng, 1 μg, 10 μg, 100 μg, 200 μg, 300 μg, 400 μg, 500 μg, 600 μg, 700 μg, 800 μg, 900 μg, 1 mg, 10 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg 1 g, 2 g, 3 g, 4 g, or 5g. In some cases, nucleic acid may be at most about 1 pg, 10 pg, 100 pg, 1 pg, 10 pg, 100 pg, 200 pg, 300 pg, 400 pg, 500 pg, 600 pg, 700 pg, 800 pg, 900 pg, 1 ng, 10 ng, 100 ng, 200 ng, 300 ng, 400 ng, 500 ng, 600 ng, 700 ng, 800 ng, 900 ng, 1 μg, 10 μg, 100 μg, 200 μg, 300 μg, 400 μg, 500 μg, 600 μg, 700 μg, 800 μg, 900 μg, 1 mg, 10 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1 g, 2 g, 3 g, 4 g, or 5g.

[00294] In some aspects, the pharmaceutical composition comprises about 1×10^6 to about 1×10^{15} recombinant viruses, about 1×10^7 to about 1×10^{14} recombinant viruses, about 1×10^8 to about 1×10^{13} recombinant viruses, about 1×10^9 to about 3×10^{12} recombinant viruses, or about 1×10^{10} to about 3×10^{12} recombinant viruses.

Kits

[00295] Compositions and reagents useful for the present disclosure may be packaged in kits to facilitate application of the present disclosure. In some aspects, the present method provides for a kit comprising a recombinant nucleic acid of the disclosure. In some aspects, the present method provides for a kit comprising a recombinant virus of the disclosure. The instructions could be in any desired form, including but not limited to, printed on a kit insert, printed on one or more containers, as well as electronically stored

instructions provided on an electronic storage medium, such as a computer readable storage medium. Also optionally included is a software package on a computer readable storage medium that permits the user to integrate the information and calculate a control dose.

[00296] In another aspect, the present disclosure provides a kit comprising the pharmaceutical compositions provided herein. In yet another aspect, the disclosure provides kits in the treatment of diseases such as, for example: AMD, DME, RVO, angiogenesis related diseases, cancer, autoimmune diseases, infectious disease organisms, and the like.

[00297] In one aspect, a kit comprises: (a) a recombinant virus provided herein, and (b) instructions to administer to cells or an individual a therapeutically effective amount of the recombinant virus. In some aspects, the kit may comprise pharmaceutically acceptable salts or solutions for administering the recombinant virus. Optionally, the kit can further comprise instructions for suitable operational parameters in the form of a label or a separate insert. For example, the kit may have standard instructions informing a physician or laboratory technician to prepare a dose of recombinant virus.

[00298] Optionally, the kit may further comprise a standard or control information so that a patient sample can be compared with the control information standard to determine if the test amount of recombinant virus is a therapeutic amount consistent with for example, a shrinking of a tumor. Optionally, the kit could further comprise devices for administration, such as a syringe, filter needle, extension tubing, cannula, and subretinal injector.

[00299] Recombinant viruses may be generated by any suitable means. The methods and compositions and of the disclosure provide for generation of recombinant virus through various means, including the use of transgenic cells, which may include mammalian cells, insect cells, animal cells or fungal cells.

[00300] For example, in some aspects, recombinant viruses may be generated through transfection of insect cells via recombinant baculovirus. In some cases, recombinant baculovirus may be generated as an intermediate, whereby the baculovirus may contain sequences necessary for the generation of other viruses such as AAV or rAAV2 viruses. In some cases one or more baculoviruses may be used in the generation of recombinant viruses used for the composition and methods of treatment of this disclosure. In some cases insect cells such as Sf9, High-Five or Sf21 cell lines may be used. In some cases,

cell lines may be generated using transient methods (i.e. infection with not stably integrated transgenes.) In other cases, cell lines may be generated through the generation of stable cell lines (i.e. infection with transgenes stably integrated into the host cell genome.)In other aspects, the pharmaceutical composition provided herein is manufactured using adherent human embryonic kidney 293 (HEK293) cells. In an alternative aspect, the pharmaceutical composition provided herein is manufactured using suspension-adapted HEK293 cells. In another aspect, the pharmaceutical composition provided herein is manufactured using the baculovirus expression system (BVES) in insect cells. In some aspects, the vector is produced using herpes-helper virus. In some aspects, the vector is produced using producer-clone methods. In some aspects, the vector is produced using Ad-AAV.

[00301] Generally, any suitable method may be used in the biochemical purification of recombinant viruses for use in a pharmaceutical composition as described herein. Recombinant viruses may be harvested directly from cells, or from the culture media surrounding host cells. Virus may be purified using various biochemical means, such as gel filtration, filtration, chromatography, affinity purification, gradient ultracentrifugation, or size exclusion methods. Recombinant virus may be tested for content (i.e., identity), purity, or potency (i.e., activity) using any suitable means, before formulation into a pharmaceutical composition. Method may include but are not limited to immunoassays, ELISA, SDS-PAGE, western blot, Northern blot, Southern blot or PCR, HUVEC assays and the like.

V. Method of Treatment

[00302] In another aspect, the present disclosure provided a method for treating a pathological angiogenesis related disease, comprising administering a pharmaceutically effective amount of the pharmaceutical compositions provided herein to a human subject in need of such treatment. In some aspects, the disease is selected from the group of ocular neovascular diseases consisting of: age-related macular degeneration (AMD), wet-AMD, dry-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 and diabetic retinal edema.

[00303] In some cases, dry AMD may be treated. In some cases, dry AMD may be referred to as central geographic atrophy, characterized by atrophy of the retinal pigment

epithelial later below the retina and subsequent loss of photoreceptors in the central part of the eye. The composition and methods of this disclosure provide for the treatment of any and all forms of AMD.

[00304] In another aspect, the present disclosure provides a method for prophylactic treatment of AMD or ocular neovascular diseases as described herein, comprising administering a pharmaceutically effective amount of the pharmaceutical compositions provided herein to a human subject in need of such treatment. The present disclosure may be used to treat patients at risk of developing AMD, or presenting early symptoms of the disease. This may include treatment of eyes either simultaneously or sequentially. Simultaneous treatment may mean that the treatment is administered to each eye at the same time or that both eyes are treated during the same visit to a treating physician or other healthcare provider. It has been documented that patients have a higher risk of developing AMD in a healthy fellow eye of an eye that presents symptoms of AMD, or in patients who have a genetic predisposition toward developing AMD. The present disclosure can be used as a prophylactic treatment in prevention of AMD in the fellow eye.

[00305] While the mechanism underlying the increased risk for the progression of ocular neovascular disease in a fellow eye is unknown, there are multiple studies in the art detailing this elevated risk. For example, in one such large scale study, of 110 fellow eyes observed that progressed to advanced AMD, choroidal neovascularization (CNV) developed in 98 eyes and foveal geographic atrophy (GA) in 15 eyes. Ophthalmologica. 2011;226(3):110-8. doi: 10.1159/000329473. Curr Opin Ophthalmol. 1998 Jun;9(3):38-46. No non-ocular characteristic (age, gender, history of hypertension or smoking) or ocular feature of the study eye at baseline (lesion composition, lesion size, or visual acuity) was predictive of progression to advanced AMD in this cohort. However, statistical analysis indicates that AMD symptoms of the first eye, including drusen size, focal hyperpigmentation, and nonfoveal geographic atrophy had significant independent relationships in assessing risk of developing of AMD in the fellow eye. Recent studies have indicated that of ocular characteristics, genetic factors and certain environmental factors may play a role in the increased risk of developing AMD in the fellow eye. JAMA Ophthalmol. 2013 Apr 1;131(4):448-55. doi: 10.1001/jamaophthalmol.2013.2578. Given the well characterized elevated risk of AMD development in untreated fellow eyes,

there is need in the art of methods for preventing onset and subsequent vision loss due to the disease.

[00306] The term "subject," or "individual" or "patient" as used herein in reference to individuals having a disease or disorder or are suspected of having a disease or disorder , and the like. Subject, individual or patent may be used interchangeably in the disclosure and encompass mammals and non-mammals. Examples of mammals include, but are not limited to, any member of the Mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. Examples of non-mammals include, but are not limited to, birds, fish and the like. In some aspects of the methods and compositions provided herein, the mammal is a human.

[00307] The term "subject" or "individual" also includes humans suffering from the disorder or disease, age 20 and older. Unexpectedly, the present disclosure can be used in a range of patient ages. This includes younger patients not generally associated with AMD disease, which presents more frequently in patients over the age of 65. Human subjects, or patients of the disclosure may include ages at least about 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100. Human subjects, or patients of the disclosure may include ages at most about 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100.

[00308] In some aspects, the term "subject," or "individual" includes patients with varying responses to penicillin, such as resistance or sensitivity to its effects or patients who show or lack symptoms of allergic response to the drug.

A. Method of Delivery

[00309] In some aspects, the pharmaceutical composition is administered to subretinal sites using any direction method. In some cases, the delivery method may be by injection, such as those described in US Pat Pub. No. 2010008170, which is incorporated by reference in its entirety. In some cases, direct administration to subretinal sites includes injection of a liquid pharmaceutical composition via syringe. In another example, direct administration may involve injection via a cannula or other suitable instrument for delivery for a vector or recombinant virus. In other examples, direct administration may comprise an implant further comprising a suitable vector for delivery of transgenes such as sFLT-1. In some cases the implant may be either directly implanted in or near the retina.

[00310] The central retina, macula, and fovea regions of the retina are unique amongst mammals to primates. Furthermore, there are distinct differences in the anatomy and subsequent pathogenesis of AMD between primate and humans. The central retina is the area of the retina surrounding the posterior pole between the vascular arcades of a primate eye, which includes the fovea, macula, and surrounding area. The macula is near the center of the retina and has a diameter of approximately 1.5 mm. This area contains the highest concentration of both rod and cone photoreceptors. At the center of the macula is the fovea, a small pit that contains the largest concentration of cone photoreceptors. The macula and fovea regions of the retina also contain underlying RPE cells. These regions of the retina are responsible for perception of fine detail (acuity) and color. As this region is responsible for the most important part of human vision (fine vision), safe and effective targeting of the vector to the subretinal space of the macula and fovea is desired. In some cases, a pharmaceutical composition of the disclosure is administered in the central retina. In some cases, it is administered in the central retina outside the fovea.

[00311] Briefly, the general method for delivering a vector to the subretinal space of the macula and fovea may be illustrated by the following brief outline. This example is merely meant to illustrate certain features of the method, and is in no way meant to be limiting.

[00312] Generally, the vector can be delivered in the form of a suspension injected intraocularly (subretinally) under direct observation using an operating microscope. This procedure may involve vitrectomy followed by injection of vector suspension using a fine cannula through one or more small retinotomies into the subretinal space.

[00313] Briefly, an infusion cannula can be sutured in place to maintain a normal globe volume by infusion (of e.g. saline) throughout the operation. A vitrectomy is performed using a cannula of appropriate bore size (for example 20 to 27 gauge), wherein the volume of vitreous gel that is removed is replaced by infusion of saline or other isotonic solution from the infusion cannula. The vitrectomy is advantageously performed because (1) the removal of its cortex (the posterior hyaloid membrane) facilitates penetration of the retina by the cannula; (2) its removal and replacement with fluid (e.g. saline) creates space to accommodate the intraocular injection of vector, and (3) its controlled removal reduces the possibility of retinal tears and unplanned retinal detachment.

[00314] In some aspects, the vector is directly injected into the subretinal space within the central retina, by utilizing a cannula of the appropriate bore size (e.g. 27-45 gauge), thus

creating a bleb in the subretinal space. In other aspects, the subretinal injection of vector suspension is preceded by subretinal injection of a small volume (e.g. about 0.1 to about 0.5 ml) of an appropriate fluid (such as saline or Ringer's solution) into the subretinal space within the central retina. This initial injection into the subretinal space establishes an initial fluid bleb within the subretinal space, causing localized retinal detachment at the location of the initial bleb. This initial fluid bleb can facilitate targeted delivery of vector suspension to the subretinal space (by defining the plane of injection prior to vector delivery), and minimize possible vector administration into the choroid and the possibility of vector injection or reflux into the vitreous cavity. In some aspects, this initial fluid bleb can be further injected with fluids comprising one or more vector suspensions and/or one or more additional therapeutic agents by administration of these fluids directly to the initial fluid bleb with either the same or additional fine bore cannulas.

[00315] Intraocular administration of the vector suspension and/or the initial small volume of fluid can be performed using a fine bore cannula (e.g. 27-45 gauge) attached to a syringe. In some aspects, the plunger of this syringe may be driven by a mechanized device, such as by depression of a foot pedal. The fine bore cannula is advanced through the sclerotomy, across the vitreous cavity and into the retina at a site pre-determined in each subject according to the area of retina to be targeted (within the central retina). In one aspect, administration is performed to a site outside the fovea. Under direct visualization the vector suspension is injected mechanically under the neurosensory retina causing a localized retinal detachment with a self-sealing non-expanding retinotomy. As noted above, the vector can be either directly injected into the subretinal space creating a bleb within the central retina or the vector can be injected into an initial bleb within the central retina, causing it to expand (and expanding the area of retinal detachment). In some aspects, the injection of vector suspension is followed by injection of another fluid into the bleb.

[00316] Without wishing to be bound by theory, the rate and location of the subretinal injection(s) can result in localized shear forces that can damage the macula, fovea and/or underlying RPE cells. The subretinal injections may be performed at a rate that minimizes or avoids shear forces. In some aspects, the vector is injected over about 15-17 minutes. In some aspects, the vector is injected over about 17-20 minutes. In some aspects, the vector is injected over about 20-22 minutes. In some aspects, the vector is injected over about 1 minute or over about 1-3 minutes or in less than one minute. In some aspects, the

vector is injected at a rate of about 35 to about 65µl/min or 65 µl/min to about 150 µl/min. In some aspects, the vector is injected at a rate of about 35µl/min. In some aspects, the vector is injected at a rate of about 40 µl/min. In some aspects, the vector is injected at a rate of about 45 µl/min. In some aspects, the vector is injected at a rate of about 50 µl/ml. In some aspects, the vector is injected at a rate of about 55 µl/min. In some aspects, the vector is injected at a rate of about 60 µl/ml. In some aspects, the vector is injected at a rate of about 65 µl/min. In some aspects, the vector is injected at a rate of about 100 µl/min. One of ordinary skill in the art would recognize that the rate and time of injection of the bleb may be directed by, for example, the volume of the vector or size of the bleb necessary to create sufficient retinal detachment to access the cells of central retina, the size of the cannula used to deliver the vector, and the ability to safely maintain the position of the cannula of the disclosure.

[00317] One or multiple (e.g. 2, 3, or more) blebs can be created. Generally, the total volume of bleb or blebs created by the methods and systems of the disclosure cannot exceed the fluid volume of the eye, for example about 4 ml in a typical human subject. The total volume of each individual bleb is preferably at about 0.1 – 0.2 ml. One of ordinary skill in the art will appreciate that in creating the bleb according to the methods and systems of the disclosure that the appropriate intraocular pressure must be maintained in order to avoid damage to the ocular structures. The size of each individual bleb may be, for example, about 50µl to about 100µl, about 50µl to about 200µl, about 0.1 to about 0.2 ml, about 0.1 to about 0.3 ml, or > 0.3 ml.

[00318] In order to safely and efficiently transduce areas of target retina (e.g. the central retina) outside the edge of the original location of the bleb, in some cases it may be desirable to manipulate the bleb to reposition the bleb to the target area for transduction. Manipulation of the bleb can occur by the dependency of the bleb that is created by the volume of the bleb, repositioning of the eye containing the bleb, repositioning of the head of the human with an eye or eyes containing one or more blebs, and/or by means of a fluid-air exchange. This is particularly relevant to the central retina since this area generally resists detachment by subretinal injection.

[00319] In some aspects fluid-air exchange is utilized following subretinal injection; fluid from the infusion cannula is temporarily replaced by air, e.g. from blowing air onto the surface of the retina. As the volume of the air displaces saline fluid from the vitreous cavity, the bleb is kept in place without efflux into the vitreous cavity. By positioning the

eye globe appropriately, the bleb of subretinal vector in some cases can be manipulated to involve adjacent areas (e.g. the macula and/or fovea). In some cases, the mass of the bleb is sufficient to cause it to gravitate, even without use of the fluid-air exchange. Movement of the bleb may be further be facilitated by altering the position of the human subject's head, so as to allow the bleb to gravitate to the desired location in the eye. Once the desired configuration of the bleb is achieved, fluid is returned to the vitreous cavity. The fluid is an appropriate fluid, e.g., fresh saline. Generally, the subretinal vector may be left in situ without retinopexy to the retinotomy and without intraocular tamponade, and the retina will spontaneously reattach within about 48 hours.

[00320] Subretinal administration of AAV-2 for treatment of an ocular disease has been demonstrated in treatment of the rare genetic disease, Leber's Congenital Amaurosis ("LCA"). The pathology of LCA and the LCA patient population are different from those of wet-AMD and therefore it was not expected that treatment of wet AMD with gene therapy, and in particular, with AAV-2, would be safe and effective prior to the rAAV.sFLT clinical study. Specifically, LCA is a degenerative genetic disease caused by insufficient expression of the retinal protein RPE-65. It causes slow deterioration of vision in babies and young children that leads to total blindness by young adulthood, generally prior to age 25 to 30. By contrast, as described here previously, wet AMD is caused by growth of new blood vessels in the retina late in life, generally beginning between age 65 - 75. The presence of new vessels raises the concern that AAV particles, the transgene or the transgene product, would be transported outside the eye in greater amounts than was shown in the LCA study. Additionally, the immune system and immune response to foreign substances changes as patients age creating uncertainly prior to study results disclosed in Example 12 that treatment of wet AMD with a viral vector such as rAAV.sFLT-1 would be safe and effective.

B. Effect of Treatment

[00321] In some aspects, a single injection of the pharmaceutical composition of the present disclosure into the affected eye not only has the benefits of the Lucentis® treatment, but may also require only one single injection.

[00322] The pharmaceutical composition of the present disclosure can stop leakage in existing blood vessels and can inhibit further new vessel formation in the subretinal space of patients suffering from CNV secondary to AMD for at least 18 months, and in some aspects the activity continues for 3-5 years. Inhibition of leakage and new vessel formation prevents the development of blindness in affected patients.

[00323] In some aspects, the sFLT-1 protein levels in the vitreous of said 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, 500 – 700 pg/ml, 700 – 1,000 pg/ml, 1,000 – 1200 pg/ml, 1200 – 1,500 pg/ml, 1,800 – 2000 pg/ml. In some cases, protein levels in the vitreous of the human subject is at least about 100, 200, 300, 400, 500, 600, 700. 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300 or 2400 pg/ml. In some cases, protein levels in the vitreous of the human subject is at most about 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300 or 2400 pg/ml.

[00324] In some cases, protein "levels" may refer to any quantity or relative quantity of protein. In some cases, level may be measured as a concentration (e.g. pM, nM, uM etc.), a molality (e.g. m), as a mass (e.g. pg, ug, ng etc.) or any suitable measurement. In some cases, a unitless measurement may indicate a level.

[00325] In some cases, protein levels may be measured at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 0.8, 0.9, 1.0, 2, 3, 4, 5, 6, 7, 14, 21 or 30, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350 or 365 days after administering said pharmaceutical composition. In some cases, protein levels may be measured at most about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 0.8, 0.9, 1.0, 2, 3, 4, 5, 6, 7, 14, 21 or 30, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350 or 365 days after administering said pharmaceutical composition. In some cases, protein levels are measured at least 72 hours after administering said pharmaceutical composition.

[00326] Administration of the pharmaceutical composition of the present disclosure general leads to no side effects or adverse events.

[00327] In some aspects, no vector is detected in the human subject's tear, blood, saliva or urine samples 7, 14, 21 or 30 days after administering said pharmaceutical composition. In some aspects, the presence of the viral vector is detected by qPCR or ELISA as known in the art.

[00328] In some cases, no vector is detected in the human subject's tear, blood, saliva or urine samples at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 0.8, 0.9, 1.0, 2, 3, 4, 5, 6, 7, 14, 21 or 30, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350 or 365 days after administering said pharmaceutical composition. In some cases, no vector is detected in the human subject's tear, blood, saliva or urine samples at most about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 0.8, 0.9, 1.0, 2, 3, 4, 5, 6, 7, 14, 21 or 30, 50, 75, 100, 125, 150, 175, 200,

225, 250, 275, 300, 325, 350 or 365 days after administering said pharmaceutical composition. In some cases, no vector is detected in the human subject's tear, blood, saliva or urine samples are measured at least 72 hours after administering said pharmaceutical composition.

[00329] In some aspects, the human subject shows no clinically significant retinal toxicity as assessed by serial ophthalmic examinations over at least about a 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 month months period. In some aspects, the human subject shows no clinically significant retinal toxicity as assessed by serial ophthalmic examinations over at most about a 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 month months period.

[00330] In some aspects, no superficial, anterior segment or vitreous inflammatory signs are present in the human subject over least a two months period. In some cases, 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.

[00331] In some aspects, no inflammatory signs are seen including a cytotoxic T cell response within about a 10% of normal range following administering step. In some aspects, there is no increase in T-cell response as measured by ELISpot. In some aspects, T cells do not express HLA-DR or Ki67, and do not develop an activated effector phenotype, as described in Lai *et al.* 2011; Gene Therapy, which is herein incorporated by reference in its entirety. In some aspects, no inflammation of the vitreous is observed by biomicroscopy (BE) and indirect opthalmoscopy (IOE) following the administering step. In some aspects, trace inflammation of the vitreous that resolved within 10 days is observed by biomicroscopy (BE) and indirect opthalmoscopy (IOE) following the administering step. In some aspects, the human subject does not require rescue treatment at least 120 days post administration. In some aspects, the human subject does not require rescue treatment for at least 30 days, at least 60 days, at least 90 days, at least 120 days at least 180 days, at least 270 days or at least 365 days after administration.

[00332] As used herein, rescue treatment refers to an administration of a dose of a VEGF inhibitor after the initial administration of the pharmaceutical composition described in the present disclosure. A rescue treatment is administered to boost the amount of VEGF inhibition in the eye patient in order to arrest or reverse signs and symptoms of disease progression. The decision to administer a rescue treatment may be based on

predetermined diagnostic criteria, as in the clinical study described in Example 12, or on a physcian's clinical judgment that signs of active disease are present in a patient.

[00333] In some aspects, 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 120 days post administration. In some aspects, 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 30 days, at least 60 days, at least 90 days, at least 120 days at least 180 days, at least 270 days or at least 365 days after administration. In some aspects, there is no evidence of visual acuity loss, IOP elevation, retinal detachment, or any intraocular or systemic immune response in said human subject at most 30 days, at least 60 days, at least 90 days, at least 120 days at least 180 days, at least 270 days or at least 365 days after administration.

[00334] In some aspects, a patient's best corrected visual acuity (BCVA) improves by 1, 2 3, 4, 5 or more lines.

[00335] In some aspects, a reduction in neovascularization as assessed by Fluorscein Angiography (FA) follows the administering step.

[00336] In some cases, retinal thickness may be measured to examine the effects of treatment. In some cases, the central retinal thickness of the human subject does not increase by more than 50 microns, 100 microns, or 250 microns within 12 months following treatment with the pharmaceutical composition of the disclosure. In some cases, the central retinal thickness of the human subject decreases by at least 50 microns, 100 microns, 200 microns, 250 microns, 300 microns, 400 microns, 500 microns, 600 microns within 3 months, 6 months or 9 months 12 months following treatment with the pharmaceutical composition of the disclosure. The decrease in the central retinal thickness of the human subject may be measured comparing the central retinal thickness at point in time to a baseline measurement taken at or within 1, 3, 7 or 10 days of the administration of the pharmaceutical composition of the disclosure.

C. Combination Treatment with VEGF inhibitors

[00337] In some aspects, the method further comprises administering to the human subject a pharmaceutically effective amount of a VEGF inhibitor.

[00338] In some aspects, the VEGF inhibitor comprises an antibody against VEGF or a functional fragment thereof. In some aspects, the VEGF inhibitor comprises ranibizumab. In other aspects the VEGF inhibitor is a soluble receptor, fusion protein, or fragment thereof, such as aflibercept or sFLT01. In some aspects, the pharmaceutical composition

is administered at least 1, 2, 3, 4, 5, 6, 7, or 8 days after the administering of said VEGF inhibitor. In some aspects, the pharmaceutical composition is administered at most 1, 2, 3, 4, 5, 6, 7, or 8 days after the administering of said VEGF inhibitor. In some aspects, the pharmaceutical composition is administered within 90 days after the administering of said VEGF inhibitor.

[00339] In some aspects, the patient is treated under a protocol such as outlined in FIG. 13. After the protein expressed by the recombinant virus is expressed at a suitable level, (or "on"), the patients are followed with criteria-based re-treatment:

a. If disease recurs, ranibizumab re-treatment is allowed

b. Expect 5-8 re-treatments per year with control group

c. In treatment group, expect equivalent vision with substantial decrease in number of re-treatments.

[00340] The patient is eligible for re-treatment if signs of active CNV are present:

a. Based upon objective criteria as evaluated by masked personnel (technician and ophthalmologist)

b. Re-treatment criteria are based upon substantial experience with "as needed" (PRN) treatment in previous trials with anti-VEGF agents.

[00341] Re-treatment is warranted based on signs of active disease; such as:

a. >10 Early Treatment Diabetic Retinopathy Study (ETDRS) letter loss from human subject's previous visit (attributable to retinal causes), OR a decrease of >5 ETDRS letters from previous visit in conjunction with patient perception of functional loss;

b. Any increased, new, or persistent subsensory, sub-Retinal Pigment Epithelial (RPE), or intraretinal fluid on OCT;

c. Signs of increased CNV leakage via FA.

[00342] In some aspects, the VEGF inhibitor is administered for at least 1 time prior to administering the said pharmaceutical composition and an additional 1 or 2 times at about 30 day intervals following said administration to prevent disease progression while protein expression increase to suitable levels. In some aspects, the VEGF inhibitor is administered for at least 2 times prior to administering said pharmaceutical composition. In some aspects, the VEGF inhibitor is administered over a period of 6 to 7 weeks following administration of said pharmaceutical composition.

[00343] In some aspects, the frequency of administration of VEGF inhibitor is reduced by less than a year or stopped altogether.

[00344] In some aspects, the present disclosure is used after 3 or more treatments of VEGF inhibitors. In some aspects, the present disclosure is used after observation that AMD patients show no improvement in BCVA after use of other VEGF inhibitors.

D. Other Combination Treatments

[00345] In another preferred aspect, treatment of a patient comprises administration one or more of the pharmaceutical compositions provided herein, in conjunction with other therapies, for example, chemotherapy, radiation, surgery, anti-inflammatory agents, selected vitamins and the like. The other agents can be administered, prior to, after or co-administered with the pharmaceutical compositions.

[00346] Aspects of the disclosure may be practiced without the theoretical aspects presented. Moreover, the theoretical aspects are presented with the understanding that Applicants do not seek to be bound by the theory presented.

[00347] While preferred aspects of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such aspects are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the aspects of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

[00348] The effective dose of the nucleic acid will be a function of the particular expressed protein, the particular disease to be targeted, the patient and his or her clinical condition, weight, age, sex, etc.

EXAMPLES

[00349] It will be understood by those of skill in the art that numerous and various modifications can be made to yield essentially similar results without departing from the spirit of the present disclosure. All of the references referred to herein are incorporated by reference in their entirety for the subject matter discussed. The following examples are included for illustrative purposes only and are not intended to limit the scope of the disclosure.

[00350] It must be explained, if not specified, that the percentage of following examples are all weight percent content wt %.

Example 1

rAAV.sFlt-1

[00351] One example recombinant virus is rAAV.sFlt-1. It encodes a vector and a human form of the truncated, soluble VEGF receptor 1 (sFLT-1). The vector is a recombinant, replicative-deficient adeno-associated viral (rAAV) vector, of serotype 2.

[00352] The rAAV.sFlt-1 was manufactured under Good Manufacturing Practices (cGMP). At the manufacture site, the final product was aliquoted into sterile, low-virus-binding microcentrifuge tubes (individually wrapped, low-retention, sterilised flat cap vials) according to the protocol requirements (i.e. 200 □l of 1 X 10^{10} or 1 X 10^{11} viral genomes) and stored at -80□C to await final product release. Each vial contained enough vector for use in a single patient (100 □l to be administered).

[00353] The recombinant virus, rAAV.sFlt-1, is a recombinant adeno-associated virus 2 (rAAV2) vector carrying the soluble VEGFR receptor 1 (VEGFR1) or sFLT-1 driven by the human cytomegalovirus (CMV) promoter. The rAAV.sFlt-1 vector and intact AAV2 genome used as the backbone was prepared as described in Lai et. al. Gene Therapy 2002 vol. 9 (12) 804-813). The rAAV2 vector is devoid of viral coding sequences, i.e., rep and cap have been replaced with an expression cassette for the therapeutic gene. The active moiety of rAAV.sFlt-1 is sFlt-1. sFLT-1 is the soluble truncated form of the vascular endothelial growth factor receptor 1 (VEGFR1 or Flt-1) which occurs naturally. sFLT-1 is the only known endogenous specific inhibitor of VEGF. sFLT-1 is generated by alternative splicing and it lacks the membrane-proximal immunoglobulin-like domain, the transmembrane spanning region and the intracellular tyrosine-kinase domain. Hence, it contains only the first six extracellular immunoglobulin-like loops followed by 31 unique amino acid residues. sFLT-1 was first identified in human umbilical vein endothelial cells (HUVEC), but it has since been found to occur naturally in the placenta and circulating systematically in pregnant women. The sFLT-1 used in generating rAAV.sFlt-1 contains an open reading frame encoding only the first six extracellular immunoglobulin-like domains of the full length membrane-spanning FLT-1, followed by a unique 31-amino acid long C-terminal extension, representing the alternatively splices, secreted soluble FLT-1 isoform described earlier.

[00354] While the ITR has been shown to possess mild promoter activity, for maximum levels of transgene expression, the cassette generally includes a promoter/enhancer combination, a small intron sequence, the cDNA of the therapeutic gene, and a

polyadenylation signal. In rAAV.sFlt-1, the human CMV major immediate early gene enhancer / promoter and a chimeric intron were placed upstream of the sFLT-1 cDNA. A simian virus 40 polyadenylation (SV40 poly A) signal was placed downstream of the sFLT-1 cDNA.

[00355] Binding of sFLT-1 to VEGF *in vitro* has been widely demonstrated. The ability of sFLT-1 to inhibit VEGF-driven angiogenesis has attracted considerable attention for its potential clinical application, but no evidence of efficacy or suitability in humans was shown prior to the clinical study of rAAV.sFlt-1 described in Example 12. The angiostatic activity of sFLT-1 results from inhibition of VEGF by two mechanisms: i) sequestration of VEGF, to which it binds with high affinity, and ii) formation of inactive heterodimers with membrane-spanning isoforms of the VEGF receptors Flt-1 and KDR/Flk-1.

Nucleotide sequence and diagram of plasmid vector used to generate rAAV.sFlt-1

[00356] rAAV.sFlt-1 was generated by triple transfection of human embryonic kidney 293 cells with DNA from the pSSV.CI.hsFlt-1 plasmid vector and helper plasmids, as is known in the art (Xiao et al., 1998. J Virololgy, 72(3): 2224-2232). rAAV.sFlt-1 was purified using a sequential process of nuclei isolation, density gradient centrifugation and heparin sulfate affinity column chromatography. A diagrammatic representation of the sFLT-1 plasmid vector is given in FIG. 1.

Formulation

[00357] rAAV.sFlt-1 was formulated in sterile phosphate buffered saline (pH7) at 2 concentrations: 1 X 1010 vector genome / 100 uL (low dose) and 1 X 1011 vector genome / 100 uL (high dose) in sterile low-virus-binding microcentrifuge tubes. The formulation is preservative-free and is for one-thaw, single use by subretinal injection only.

rAAV(bv).sFlt-1

[00358] A second example recombinant virus is rAAV(bv).sFlt-1. rAAV(bv).sFlt-1 is a recombinant, replicative-deficient adeno-associated viral (rAAV) vector, of serotype 2 that is produced using a baculovirus expression system (BEVS) in Sf9 insect cells, and encodes a human form of the truncated, soluble VEGF receptor 1 (sFLT-1). The vector was produced using infection in Sf9 cells with two recombinant baculovirsues, Bac-inRep-inCap and Bac-sFlt-1. Bac-sFlt-1 was derived from bacmid DNA that was generated from transformation of electrocompetent cells with an 8.7 kb plasmid, AVA01-pFB-CMV-sFlt, which was cloned from the Frag001m-BHKan and the plasmid backbone

V109-pFB-AAV-CMV-SV40pA-Kan using standard molecular biology techniques, as described in Maniatis et al., and as further described below. Frag001m was formed from the following sequential nucleic acide elements which were chemically synthesized by Blue Heron Biotech, LLC (Bothell, WA) and cloned into a BHKan backbone: an ITR (AAV serotype 2), CMV-IE promoter, chimeric intron, 5' untranslated region (UTR), sFlt-1 coding sequence, SV40 polyA region, ITR (AAV serotype 2). The plasmid V109-pFB-AAV-CMV-SV40pA-Kan was obtained from Virovek, Inc. (Hayward, CA). The plasmid contained a kanamycin antibiotic resistance gene, a ColE1 origin and a recombinant AAV cassette, which contained a CMV-IE promoter, an intron, multiple cloning sequences and a SV40 polyA region, flanked by inverted terminal repeats (ITRs) from AAV serotype 2. This rAAV cassette was flanked by a gentamicin resistance gene and Tn7L attachment sites. AVA01-pFB-CMV-sFlt did not contain a T7 RNA polymerase promoter or other prokaryotic regulatory sequence. Bac-inRep-inCap is a recombinant baculovirus containing expression cassettes for rep and cap genes from AAV serotype 2.

rAAV(bv).sFlt-1 Production in Baculovirus

[00359] rAAV(bv).sFlt-1 was produced in baculovirus according to the methods described in US Patent Application 12/297,958 and more specifically as follows: Sf9 cells were grown at 28° C to about 107 cells/ml in SF900 II SFM media containing 100 units/ml of penicillin and 100 μg/ml streptomycin, and diluted to about 5×106 cells/ml prior to infection. Bac-inRep-inCap and Bac-sFlt-1, each at m.o.i. of one were used to infect the cells at 28°C for 3 days to produce AAV type 2 vectors. After 3 days of infection, cell pellets were collected by centrifugation at 2,000 rpm for 15 min in a tabletop centrifuge. The cell pellets were lysed in lysis buffer as described by Urabe et al., Hum Gene Ther. 1; 13(16):1935-43 (2002) and cellular nucleic acids (DNA and RNA) were digested by benzonase (Sigma, St. Louis, Mo.). The cell lysates were cleared by centrifugation at 8,000 rpm for 30 min in an Avanti J-25 centrifuge (Beckman, Fullerton, Calif.) and then loaded onto an SW28 centrifuge tube containing 5 ml of 1.55 g/cc, and 10 ml of 1.32 g/cc of CsCl solutions. After centrifugation at 28,000 rpm for about 16 hours at 15° C., the rAAV-containing fraction was collected by puncturing the centrifuge tube using a syringe needle and subjected to a second round of CsCl ultracentrifugation. The rAAV-containing fraction was collected again by puncturing the centrifuge tube using a syringe needle and dialyzed in PBS buffer to remove the salts and detergents. Vector

titers were determined by quantitative real-time PCR assay according to manufacturer's protocol (Applied Biosystems, Foster City, Calif.).

Example 2

In vitro inhibition of VEGF-induced endothelial cell proliferation

[00361] Studies were performed to assess VEGF-induction of human umbilical vein endothelial cell (HUVEC) proliferation and to determine whether VEGF-induced HUVEC proliferation would be inhibited by rAAV-mediated sFLT-1. The presence of sFLT-1 in transduced cells was first confirmed by Western blot analysis of conditioned media (FIG 2, panel a). Conditioned medium from rAAV.sFlt-1-transduced and rAAV.gfp-transduced 293 cells were added to VEGF-treated HUVECs in increasing dilutions. A control starvation medium (normal HUVEC growth medium without bovine endothelial growth factor) only was also included. Heparin was added to each well at 100 μg/mL. The relative VEGF-induced proliferation of HUVECS treated with VEGF and the different conditioned media was assayed by addition 25 μL of 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrozolium bromide (MTT, 5 mg / mL, Sigma) to each well for 4 hours at 37°C. The secreted sFLT-1 encoded by the rAAV vector in 40 μL of conditioned medium from rAAV.sFlt-1-transduced 293 cells was confirmed to inhibit VEGF-induced proliferation of HUVECS by 32%. Doubling the volume of conditioned medium resulted in complete inhibition with cell growth equivalent to basal levels similar to culture in starvation medium (FIG. 2, panel b).

In vitro Assessment of rAAV.sFt-1 Vector Potency

[00362] Studies were performed to assess the potency of AAV vectors encoding the recombinant human sFlt-1 gene by quantifying human sFlt-1 protein expression of transduced human embryonic kidney 293 (HEK293) cells by ELISA. Human embryonic kidney 293 cells were obtained from the American Type Culture Collection (Rockville, MD, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Grand Island, NY, USA) with 10% Fetal bovine serum (FBS, GIBCO) and 1X Penicillin-Streptomycin-Glutamine. All cultures were maintained at 37°C and 5% CO2 in a humidified atmosphere.

[00363] The HEK293 cells were seeded at 8E4 or 1.5E5 cells/24 well and transduced at 60-90% confluency with the recombinant AAV vectors at a multiplicity of infection (MOI) ranging from $1x10^3$-$1x10^6$ in DMEM medium supplemented with 2% FBS. After 72 hours, post-transduction, conditioned media were collected. Aliquots of the

conditioned media were prepared for ELISA using reagents and according to standard instructions from the R&D Systems SVR100B Quantikine ELISA Human sVEGF R1/ sFlt-1 kit. (R&D Systems, Minneapolis, MN). Samples, standards and controls were prepared according to the ELISA kit instructions with the R&D Systems ELISA reagents and then transferred to an ELISA plate pre-coated with an antibody to sVEGF R1/ sFlt-1 and incubated for two hours at room temperature on a horizontal orbital microplate shaker. After incubation, anti-sVEGF R1 Conjugate (two hours), substrate solution (30 minutes) and stop solution were sequentially applied to each well with aspiration and wash steps between each according to standard ELISA assay procedures. The optical density (OD) of the samples, standards and controls was measured within 30 minutes of stopping the substrate reaction with an ELISA plate reader. The concentration of sFlt-1 in pg/mL was calculated using SoftmaxPro software using the OD measurements from the ELISA plate reader..

[00364] Results of the studies for rAAV.sFlt-1 and rAAV(bv).sFlt-1 are presented in FIG. 25A and FIG. 25B. The concentration of sFlt-1 protein expressed by HEK293 cells 72 hours after transduction with rAAV.sFlt-1 and rAAV(bv).sFlt-1 ranged from 100-1,000 pg/mL at an MOI of $1x10^4$, 100-10,000 pg/mL at an MOI of $1x10^5$ and 1,000-10,000 pg/mL at an MOI of $1x10^6$.

Example 3

rAAV.sFlt-1 Studies in Mice

[00365] Transgenic mice (trVEGF029) with slow, but stable retinal neovascularization induced by transgenic expression of human VEGF from photoreceptor cells were used as a model for retinal neovascularization. Two separate studies with these mice have been conducted.

[00366] In the first mouse study, 13 transgenic mice were assessed for ocular neovascular changes before and after administration of the rAAV.sFlt-1 vector ($1 X 10^{11}$ vector particles) in one eye and control vector in the contralateral eye. Eyes were assessed for neovascular changes using fluorescein angiography at one, three and eight months after injection. The extent, intensity and stage of neovascularization were graded (0 - 4) by three observers, masked to the treatment received in the eyes examined. There was a statistically significant overall reduction in the neovascular grading from a median grade of '3' (before injection) to a median grade of '1' at one month after injection ($P = 0.012$). This reduction was maintained at three months (median = 1; $P = 0.001$) and at eight

months (median = 1; P = 0.001) after injection with rAAV.sFlt-1. Injection of rAAV.sFlt-1 vector resulted in the long-term (at least eight months) regression of neovascular vessels in 85% (11 of 13) of treated eyes compared to 8% (1 of 13) in the control vector-treated eyes.

[00367] Histological examination of the eyes in this preclinical study revealed that disturbance or loss of photoreceptors was significantly ($P < 0.01$) more pronounced in control vector-injected eyes compared to eyes injected with rAAV.sFlt-1. Expression of sFLT-1 was also confirmed by reverse transcriptase-polymerase chain reaction analysis of tissue samples; mRNA for sFLT-1 was detected in all four eyes tested. No rAAV.sFlt-1 vector-specific adverse effects were noted in the eye injected with rAAV.sFlt-1 when compared to the eye injected with the control (rAAV.gfp) vector.

[00368] In the second study, conducted in trVEGF02 transgenic mice, the aim of the study was to determine whether subretinal injection of rAAV.sFlt-1 resulted in any cell-mediated immune responses that could negatively impact on long-term expression of sFLT-1 or cause immune response-associated damage to the retina. In this study, 50 trVEGF02 transgenic mice were given subretinal injections of rAAV.sFlt-1 (8 X 109 viral particles) or phosphate-buffered saline (PBS) in one eye. The retinas of 30 mice from either the rAAV.sFlt-1 or control treatment groups were then assessed at one week and one month post-injection for the presence of immune cells (leucocytes, macrophages and B- and T-lymphocytes). Flow cytometric examination of the posterior eye cup showed that at one week post-injection there was a statistically significant increase in CD45+ leucocytes (6.6-fold increase compared to control; $P < 0.05$) and CD11b+ macrophages (5.7-fold increase compared to control; $P < 0.036$). However, there were no differences in CD19+, CD8+ and CD4+ (B- and T-lymphocytes) at this time point. At one month post-injection, there were no differences in cell numbers between leucocyte subsets (i.e. CD45+, CD19+, CD11b+, CD8+ or CD4+ cells) in the mouse eyes treated with rAAV.sFlt-1 or the PBS control, suggesting that the infiltration of leucocytes and macrophages was transient. Flow cytometric evaluation of lymphocyte subsets of the spleens from these mice at the one-week and one-month time points showed no significant differences in the numbers of lymphocytes. This finding suggests that there was no systemic immune response observed, albeit a transient, localized immune response had been shown in the retina.

[00369] In this second study, histological examination of the eyes from five of the mice injected with either rAAV.sFlt-1 or PBS revealed no observable immune-response associated destruction or sequelae in the retinas of any of the mice examined.

[00370] To assess the impact of rAAV.sFlt-1 on the level of neovascularization in this transgenic mouse model (trVEGF02) of retinal neovascularization, the retinas of the mice injected with either rAAV.sFlt-1 or PBS were also graded independently by two different assessors at two months after treatment. Overall, there was a significant reduction in mean neovascularization grades (before injection: 1.46 ± 0.58; after injection: 0.81 ± 0.57; $P < 0.00015$) in the rAAV.sFlt-1-injected eyes whereas there was a significant increase in mean neovascularization grades (before injection: 1.08 ± 0.56; after injection: 1.63 ± 0.96; $P < 0.018$) in the PBS control-injected eyes.

[00371] The findings from this second mouse study clearly indicate that treatment with rAAV.sFlt-1 appeared to reverse the progressive increase in neovascularization observed in this mouse model of retinal neovascularization and AMD. Furthermore, only a limited, localized, inflammatory response was observed one week after subretinal injection with rAAV.sFlt-1 and resolved at one month. This immune response did not appear to compromise the long-term therapeutic efficacy of rAAV.sFlt-1 in the retina.

[00372] The transgenic mice models described in this Example 3 demonstrate that the pharmaceutical compositions disclosed herein can be used for the treatment and/or prophylaxis of other retinal vascular diseases in which VEGF inhibition is implicated. These include diabetic macular edema, diabetic retina ischemia, diabetic retinal edema, proliferative diabetic retinopathy, retinal vein occlusion, central retinal vein occlusion and branched retinal vein occlusion. In clinical studies some VEGF inhibitors, such as Lucentis, have been shown to effectively treat certain of these diseases including diabetic macular edema and retinal vein occlusion. The efficacy of rAAV.sFlt-1 demonstrated in these mouse models indicates rAAV.sFlt-1 is also effective in treating these VEGF mediated diseases.

Example 4

rAAV.sFlt-1 Study in Rats

[00373] In the rat rAAV.sFlt-1 study, two models of ocular neovascularization were used: cautery-induced corneal neovascularization and laser photocoagulation-induced choroidal neovascularization (CNV). In the corneal neovascularization model, 22 rats were injected with rAAV.sFlt-1 vector (8×10^8 viral particles) in the anterior chamber of

one eye and with control vector (rAAV.gfp) in the contralateral eye, followed by cauterization of the cornea. The eyes were then examined for neovascularization four days after cautery, using slit-lamp photography. A significantly lower rate of corneal vascularization was found in the rAAV.sFlt-1-treated eyes compared to the control-treated eyes (27% and 63%, respectively; P = 0.009). Histological examination of the eyes showed that no corneal blood vessels were observed in the majority of cauterized, rAAV.sFlt-1-tcated eyes. Histological examination also revcaled that cellular infiltration of the corneal stromal layer was more pronounced in the control vector-injected eyes compared to the rAAV.sFlt-1-treated eyes. In addition, there was obvious edema and corneal stroma swelling in the control vector-treated eyes whereas there was no evidence of significant tissue swelling in rAAV.sFlt-1-treated eyes.

[00374] In the laser photocoagulation-induced CNV model, 10 rats were injected subretinally with rAAV.sFlt-1 vector (8 X 108 viral particles) in one eye, and a control vector (rAAV.gfp) in the contralateral eye. Laser photocoagulation was used to induce CNV one month after injection. Five weeks after laser photocoagulation, eyes were examined for CNV using fluorescein angiography. Only 41% of the laser-treated areas showed leakage in the rAAV.sFlt-1 treated eyes compared to 60% in the control vector-treated eyes (P = 0.002). Sixteen weeks after laser-induced CNV, the rAAV.sFlt-1-treated eyes still showed significantly lower neovascularization than control eyes. Histological examination of the eyes in the areas immediately adjacent to the injection sites revealed a normal retinal pigmented epithelium and normal outer segments and outer nuclear layer. These findings suggested there was no obvious toxicity associated with sFLT-1 expression. Electroretinograms also indicated normal functioning of rAAV.sFlt-1-treated eyes. Most of the rAAV.sFlt-1 and control vector-treated laser lesions developed subretinal cellular membranes. However, the lesions in eyes treated with rAAV.sFlt-1 generally had less proliferating endothelial cells, reflecting the fluorescein angiography findings, and indicating that the rate of angiogenesis (i.e. neovascularization) was reduced in rAAV.sFlt-1-treated eyes.

rAAV.sFlt-1 and rAAV(bv).sFlt-1 Study in Rat Model of Diabetes

[00375] To further assess the safety and efficacy of rAAV.sFlt-1 and rAAV(bv).sFlt-1 for the treatment of diabetic retinopathy (DR) and diabetic macular edema (DME), an experiment in a rat model of diabetes is conducted.

[00376] Vision loss in diabetic patients is mediated by inflammation, leading to the eventual breakdown of the blood-retinal-barrier and subsequent vascular leakage, resulting in macular edema. The streptozotocin (STZ)-diabetic rat model displays a well-characterized pattern of vascular leakage, in which VEGF is strongly upregulated as early as 2 weeks. (Miyamoto, K., et al. Proc Natl Acad Sci USA 96, 10836-10841 (1999). Current approaches to treating animal models of DR demonstrate only a partial resolution of vascular leakage.

[00377] Diabetes is induced in Brown Norway rats by intraperitoneal injection of streptozotocin (50 mg/kg). Diabetes is confirmed and monitored by blood glucose measurements. Rats with blood glucose > 350 mg/dl are considered diabetic. Eight days following onset of diabetes, rats are treated by subretinal injection (n = 12 eyes per group) with 5 μL containing either $1x10^{10}$ or $5x10^{10}$ vg of rAAV.sFlt-1 or rAAV(bv).sFlt-1 using established techniques as described in Chalberg, T.W. et al., Invest Ophthalmol Vis Sci 46, 2140-2146 (2005). AAV2.GFP ($5x10^{10}$ vg) and vehicle are be injected as controls. Non-diabetic and diabetic no-treatment groups are also used as controls.

[00378] The effect of the rAAV(bv).sFlt-1 expressing sFLT-1 on vascular leakage is measure at 60 days. Retinal vascular leakage is measured by the FITC-albumin leakage method following the injection using the FITC-conjugated albumin as tracer. The FITC-albumin leakage method directly measures the leakage of FITC-albumin leaking into the retina from the circulation and is a commonly used method to measure retinal vascular permeability. Retinal vascular leakage in injected eyes will be compared to non-diabetic controls, untreated and vehicle-treated diabetic eyes, and wildtype AAV serotypes 2 and 8.

[00379] Results: rAAV(bv).sFlt-1 expressing sFLT-1 reduces vascular leakage in the STZ-diabetic rat whereas injection of AAV2.GFP and other controls does not.

Example 5

rAAV.sFlt-1 Study in Monkeys

[00380] The efficacy and safety of rAAV.sFlt-1 was also examined in a nonhuman primate (macaque) model of AMD using laser photocoagulation to induce CNV. One challenge in developing treatments for AMD in humans is that nonhuman primates do not develop AMD. Laser photocoagulation induced CNV simulates some symptoms of AMD, but the underlying biological process is healing of an acute injury rather than progression of a chronic disease and thus may not be predictive of the performance of any

particular treatment for CNV in humans with AMD or other CNV based diseases. Nonetheless, because human eyes are anatomically more similar to nonhuman primate eyes than nonprimate eyes, nonhuman primates are frequently studied to assess toxicity and histological response to a potential treatment or other intervention.

[00381] In the first study on nonhuman primates, five macaque monkeys were injected subretinally with rAAV.sFlt-1 (4×10^{12} viral particles) in one eye, and a control vector (rAAV.gfp) in the contralateral eye. The eye health of the monkeys was periodically assessed after subretinal injection. There was no apparent complication related directly to subretinal injection of either the control or rAAVsFlt-1 vector. A transient conjunctival irritation and vitreous haze was noted in the week following injection, which cleared by the second week. Subretinal injection was unsuccessful in the right eye of one of the monkeys; therefore this animal was not subjected to further evaluation.

[00382] Subretinal injection of 40-100 uL of rAAV suspension lifted the retina, creating a bleb that housed the vector between the pigment epithelium and the photoreceptor layer in a localized manner. This bleb self-corrected within 24 to 48 hours. Except for a minor disturbance to the retinal pigment epithelium at the point of needle penetration, no other retinal abnormalities were observed for the duration of the follow-up (3 to 17 months post-injection). No other abnormalities or adverse events were observed; at no time was retinal detachment associated with the surgery.

[00383] To assess the long-term therapeutic efficacy of rAAVsFlt-1, the four injected monkeys were then subjected to intense laser photocoagulation 16 months after treatment with the vectors. Eight lesions were induced using laser in each eye, and the eyes then monitored for CNV at two and four weeks after laser treatment. After laser photocoagulation, only three of the four monkeys were analyzable, therefore, efficacy data was collected for three animals. None of three monkey eyes treated with rAAVsFlt-1 developed CNV-related lesions and only weak fluorescein staining was observed, indicating minimum leakage / neovascularization. All contralateral eyes treated with control vector developed CNV-related lesions.

[00384] In a follow-up study aimed at assessing the safety and toxicity of rAAV.sFlt-1 injected into the subretinal space, eight monkeys were used: five were injected in their left eyes with rAAV.sFlt-1, two injected in their left eyes with rAAV.gfp, one injected in both eyes with recombinant Flt-1 protein and one was kept as uninjected control. The monkeys were examined preinjection and post injection by color fundus photography, fluorescein

angiography and electroretinography. Blood was collected routinely for assaying sFLT-1 levels and peripheral blood lymphocytes were isolated for flow cytometry to assess immune cell subset response. At time of sacrifice (3, 9 and 12 months post injection), tissues were collected for i) biodistribution studies on the rAAV.sFlt-1 vector using real-time polymerase chain reaction on extracted genomic DNA; ii) hsFlt-1 protein and AAV2 capsid protein level quantitation by ELISA; and iii) histology of the eyes.

[00385] Color fundus photography, fluorescein angiography and electroretinography did not detect any adverse effect on the eye following injection. Plasma sFLT-1 level did not show any rAAV.sFlt-1 injection-related rise in level in any of the male or female monkeys examines. Except for an optic nerve sample, the rAAV.sFlt-1 sequence was not detected in the genomic DNA of any of the other tissues sampled (lymph nodes, spleen, liver, brain, brain, heart, spleen, cornea). Haematoxylin and eosin stained paraffin-embedded sections of the eyes appeared normal.

[00386] While non-human primate anatomy is more similar to human anatomy than the anatomy of smaller mammals such as mice, limitations do exist which make studies in non-human primates intriguing, but not predictive of clinical results in humans. As noted above, the study in this example uses a laser injury model in which the animal has otherwise healthy retinal tissue. The retinal tissue was not degraded over time as in disease retinal tissue nor are the disease specific pathogenic factors present. Non-human primates frequently differ from humans with respect to biodistribution, pharmacokinetics and dose dependencies, antibody titer, immune response and inflammatory response in ways that are not predictable. Additional differences include the ILM (inner limiting membrane) and the volume of the vitreous chamber, which is approximately four times larger in humans than the nonhuman primates used in this study. The human inner limiting membrane, a barrier that acts to limit transport between the retina and the vitreous, is a more a more profound and effective barrier than the ILM of a monkey.

Example 6

Safety Studies

[00387] In these studies, sFLT-1 protein was measured in the vitreous and plasma of animals using an enzyme linked immunosorbent assay kit for sFLT-1 protein detection. sFLT-1 protein level was upregulated in vitreous and eyes of animals injected with rAAV.sFlt-1. FIG. 3A shows the vitreous sFLT-1 protein level in monkey eyes injected with rAAV.sFlt-1 (left eye) and control eye injected with rAAV.gfp and uninjected eyes

(right eye). sFLT-1 protein levels were significantly higher in four out of the five rAAV.sFlt-1 injected eyes. Table 5.3.1 shows the sFLT-1 protein level in the mouse eyes that were not injected and that were injected with rAAV.sFlt-1 and enucleated at one month post injection. Overexpression of sFLT-1 in the eyes of mice and vitreous of monkeys did not have any adverse effect on their overall well-being. In monkeys, sFLT-1 overexpression in the vitreous did not have any effect on their retinal function and did not have any clinically or histologically evident toxic effects on the eyes. The significantly higher sFLT-1 protein levels in the rAAV.sFlt-1 injected eyes suggests long-term rAAV-mediated hsFLT-1 expression and supports previous data on detection of viral mRNA sequence and presence of rAAV-mediated gfp expression in monkey retina 17 months post injection.

Table 1 Summarizing hsFLT-1 protein levels in rAAV.sFlt-1-injected mouse eyes and uninjected mouse eyes at 1 month post injection.

Animal species and number of eyes	Treatment	Time post injection (week)	sFLT-1 protein level (pg/mL)
Mouse (n=1)	uninjected	NA	101.4 ± 4.8
Mouse (n=1)	uninjected	NA	91.0 ± 10.9
Mouse (n=1)	uninjected	NA	113.4 ± 6.3
Mouse (n=1)	uninjected	NA	160.2 ± 8.9
Mouse (n=1)	rAAV.sFlt-1 injected	4	1034.7 ± 44.3
Mouse (n=1)	rAAV.sFlt-1 injected	4	610.3 ± 16.3
Mouse (n=1)	rAAV.sFlt-1 injected	4	1417.2 ± 50
Mouse (n=1)	rAAV.sFlt-1 injected	4	>max

[00388] Plasma hsFLT-1 levels in the monkeys did not show any trend at the different sampling times (FIG. 3B). This suggests that the injection of rAAV.sFlt-1 did not have an obvious effect on the plasma hsFLT-1 level. The fluctuating levels did not have any effect on the well-being of the monkeys.

Table 2 Immunogenicity Studies

Species/Strain	Method of administration	Duration of dosing	Doses	GLP compliance
C57Bl/6 mice	subretinal	1, 2 and 4 weeks	8 X 10^{9} vector genomes	No
Monkeys	subretinal	12 months	8 X 10^{11} vector genomes	No

[00389] Table 2: Summary of animal strain, injection route, duration and dose of rAAV.sFlt-1 used in immunogenicity studies

Example 7

Immunogenicity studies on mice

[00390] The cellular immune response to rAAV.sFlt-1 therapy was assessed in the mouse eye one, two and four weeks post injection using flow cytometry. Infiltrating leucocytes were identified on the basis of CD45 expression and classified as monocytes/granulocytes, B cells, $CD4^+$ T cells and $CD8^+$ T cells on the basis of CD11b, CD19, CD4 and CD8 expression, respectively. The posterior eye cup was collected from five mice in each group (rAAV.sFlt-1-injected, PBS-injected, uninjected control) and pooled for analysis. As shown in FIG. 4A, there was no difference in the number of cells recovered from each group of mice over the course of this experiment. However, there was a significant increase in the number of $CD45^+$ cells one and two weeks post injection that disappeared by four weeks (FIG. 4B). Almost all of the increase seen at one week could be attributed to an increase in $CD11b^+$ cells (FIG. 4C), since there was no difference in the number of $CD4^+$, $CD8^+$, and $CD19^+$ cells (FIGS. 4D-F). At two weeks though, there was no longer a significant difference in the number of $CD11b^+$ present in the eyes of AAV.sFlt-1 injected mice; instead, there was a significant increase in the number of $CD4^+$ and $CD8^+$ T cells and a possible trend towards an increase in B cells. The number of $CD4^+$ and $CD8^+$ cells fell sharply at four weeks yet remained significantly increased compared to the PBS-injected and uninjected mice. In contrast, there was no change in the number of $CD11b^+$, $CD4^+$, $CD8^+$ and $CD19^+$ cells in the spleen during the course of this experiment (FIGS. 5A-E).

[00391] The function of the T cells infiltrating the retina was examined more closely by stimulating them with PMA/ionomycin or anti-CD3 and measuring intracellular IFN-gamma production by flow cytometry. FIG. 6 shows that compared to uninjected controls, a small proportion of both $CD4^+$ and $CD8^+$ T cells were primed to produce IFN-gamma after the injection of rAAV.sFlt-1. The frequency of IFN-gamma producing cells did not vary significantly over the course of the experiment despite an apparent increase amongst $CD8^+$ T cells on day 3 (FIG. 6B). Lower levels of IFN-gamma were measured when the T cells were restimulated with a class I MHC-restricted epitope of rAAV capsid protein and some IFN-gamma was also detected in the absence of any stimulation (data not shown). Taken together, these results indicate a small proportion of the T cells infiltrating the eyes

of rAAV.sFlt-1-injected mice had been recently activated to produce IFN-gamma, but this did not vary amongst either T cell subset during the course of this experiment.

[00392] The data presented for these experiments on the infiltration of immune cells into the eyes of AAV-sFLT-1 injected mice clearly show two waves of cell infiltration. There was an early wave of $CD11b^+$ cells at 1 week followed by a wave of $CD4^+$ and $CD8^+$ T cells at 2 weeks. Importantly, neither wave of infiltration was still present at 4 weeks, suggesting the infiltration had resolved itself. Importantly, sFLT-1 protein production was did not wane at this point, and indeed, continued to be expressed at very high levels.

[00393] The data on IFN-gamma production indicated that around 5% of the $CD4^+$ and $CD8^+$ T were recently primed, and this frequency did not vary over the course of the experiment. Hu. et al first described the breakdown of the blood-retinal barrier by activated T cells, and the data presented here is consistent with the infiltration of activated $CD4^+$ and $CD8^+$ cells. However, there was no evidence of an increase in the number of capsid-specific T cells amongst this population since restimulation with specific peptide only revealed low and levels of IFN-gamma production that did not change over the course of the experiment. Taken together, these observations suggest that the initial insult that occurred with injection of rAAV.sFlt-1 produced a short-lived wave of immune cell infiltration that resolved itself within four weeks, but failed to elicit an ongoing immune response that could harm the tissues of the eye or affect sFLT-1 expression.

Example 8

Immunogenicity studies on monkeys

[00394] Immune response following subretinal injection of rAAV.sFlt-1 or rAAV.gfp was analyzed using a panel of antibodies that would identify changes in immune cell subset populations. The results are summarized in FIG. 4. In some monkeys, very small changes in immune cell subset populations were observed but they were not statistically significant. Despite this, this was followed by a more in-depth study of circulating cells. Specifically, we assessed the possibility that either the vector (rAAV) or the inserted gene product (sFLT-1) may cause immune activation. Activation of B cells and T cells was investigated (FIG. 5 and FIG. 6). Other lymphocyte populations were also analyzed to determine whether the therapy caused any observable differences that may be indicative of direct activation or a response to activation. Analysis was conducted using a combination of classic markers (Pitcher, 2002 #129), as well as a novel phenotypic analysis described in a recently published report (Miller, 2008 #126). Using a small

subset of phenotypic markers (HLA-DR, Ki-67, and Bcl-2) we investigated whether following administration of rAAV-sFLT-1 CD4+ or CD8+ T cells and/or B cells showed signs of activation. In the studies published by Miller and colleagues, activated T cells display an activated effector phenotype characterized by the expression of the differentiation marker HLA-DR and the cell cycle associated nuclear antigen Ki-67, which is used as a marker for proliferation. Resting T cells do not express Ki-67, whereas cycling or recently divided T cells upregulate Ki-67 expression. A level of Ki-67 expression is normally detected as part of homeostatic cell cycling.

Example 9

Biodistribution of rAAV.sFlt-1

[00395] Genomic DNA was extracted from tissues collected (optic nerve, lymph node, brain, heart, lungs, spleen, liver, cornea) immediately after euthanasia of monkeys. Real time polymerase chain reaction was performed on the genomic DNA to determine whether the rAAV.sFlt-1 vector construct injected in the subretinal space would be present elsewhere. Based on comparison of Ct values between known amounts of control plasmid pssv.C1.sflt-1 DNA, the rAAV.sFlt-1 construct was found at low gene copy number in the optic nerve of one injected eye and not in any of the other tissues samples. This suggests that rAAV.sFlt-1 injected into the subretinal space remains mainly within the eye. Table 4 is a summary of the Ct values from genomic DNA extracted from monkeys that were not injected or injected with rAAV.sFlt-1- and rAAV.gfp.

Table 3: Ct values and Ct standard deviation values for the different genomic DNA and control plasmid DNA samples analyzed.

Sample ID	**Ct Mean**	**Ct Std Dev**
No DNA 0 copy	40.83970125	0.08415232
pssv.C1.hsFlt-1 (0.045ng) 6000000 copies	18.17500393	0.522299978
pssv.C1.hsFLT-1 (0.009.ng) 1000000 copies	22.5311632	0.318372962
pssv.C1.hsFLT-1 (0.0009ng) 100000 copies	26.23701276	0.183232131
pssv.C1.hsFLT-1 (0.00009ng) 10000 copies	25.2483849	0.164140658
pssv.C1.hsFLT-1 (0.000009ng) 1000copies	29.4265616	0.415926721
Control uninjected monkey		
1 LE Optic nerve	42.11	0.573
2 RE Optic nerve	43.58	0.323
3 axillary LN	45.86	1.319
4 cervical LN	N/A	N/A
5 spleen	40.71	0.093
6 liver	44.16	0.604
Monkey 999: rAAV.sFlt-1 injected, euthanized 3 mo p.i.		
7 LE optic nerve	39.13	0.137
8 RE optic nerve	42.25	0.153
9 axillary LN	40.87	0.728

Sample ID	Ct Mean	Ct Std Dev
10 submandibular LN	40.54	0.453
11 spleen	N/A	N/A
12 liver	41.23	0.388
Monkey 8294: sFLT-1 protein injected, euthanized 3 mo p.i.		
13 LE optic nerve	42.15	0.545
14 RE optic nerve	42.67	0.411
15 axillary LN	43.92	0.304
16 submandibular LN	N/A	N/A
17 spleen	N/A	N/A
18 liver	40.45	0.981
Monkey 8524: rAAV.sFlt-1 injected, euthanized 9 mo p.i.		
19 left cornea	39.72	0.975
20 right cornea	N/A	N/A
21 axillary LN	44.12	0.216
22 cervical LN	N/A	N/A
23 spleen	37.91	0.668
24 liver	41.8	0.648
Monkey 8514: rAAV.sFlt-1 injected, euthanized 12 mo p.i.		
25 right optic nerve	39.96	0.609
26 left optic nerve	28.9	0.057
27 axillary LN	40.08	0.221
28 cervical LN	41.27	0.063
29 spleen	39.22	0.196
30 liver	40.79	0.367
31 brain	41.14	0.798
32 heart	42.19	0.265
33 lungs	40.11	2.093
Monkey 8523: rAAV.sFlt-1 injected, euthanized 12 mo p.i.		
34 left optic nerve	37.27	0.838
35 right optic nerve	37.92	1.181
36 axillary LN	38.55	0.895
37 cervical LN	39.68	0.583
39 spleen	36.44	0.519
40 liver	39.94	0.768
41 8523 brain	40.29	0.397
42 8523 heart	41.28	0.877
43 8523 lungs	41.71	1.186
Monkey 8530: rAAV.sFlt-1 injected, euthanized 12 mo p.i.		
44 left optic nerve	38.52	0.777
45 right optic nerve	40.67	1.354
46 axillary LN	42.49	0.841
47 cervical LN	38.55	0.895
48 spleen	36.44	0.519
49 liver	39.94	0.768
50 brain	40.29	0.397
51 heart	41.67	1.787
52 lungs	39.29	1.474
Monkey 8532: rAAV.sFlt-1 injected, euthanized 12 mo p.i.		
53 left optic nerve	35.07	1.06
54 right optic nerve	38.14	0.665

Sample ID	Ct Mean	Ct Std Dev
55 axillary LN	40.23	1.171
56 cervical LN	40.82	0.496
57 spleen	40.09	0.195
58 liver	40.63	1.1052
59 brain	38.68	0.295
60 heart	40.04	0.685
Monkey 8297: rAAV.sFlt-1 injected, euthanized 12 mo p.i.		
61 left optic nerve	39.84	1.034
62 right optic nerve	42.17	1.247
63 axillary LN	41.19	2.174
64 cervical LN	41.38	2.040
65 spleen	39.09	1.273
66 liver	41.36	0.683
67 brain	37.84	1.243
68 heart	40.74	0.868
69 lungs	42.60	0.276

Example 10

Efficacy studies on a mouse model of retinal neovascularization

[00396] Transgenic mice generated through VEGF upregulation in the photoreceptors cells were used in the study. One eye was injected with rAAV.sFlt-1 and the contralateral eye was injected with rAAV.gfp. The extent, intensity, and stage of neovascularization were graded by masked observers based on an agreed scale. The results shown that there was a statistically significant overall reduction in neovascularization grades from a median of 3 (severe) to a median of 1 (mild) at one month post injection (P = 0.012). This low level of fluorescein leakage was maintained at three (median = 1; P = 0.001) and eight months (median 1; P = 0.001) post-rAAV.sFlt-1 injection suggesting the long-term, sustained therapeutic effect of rAAV.sFlt-1.

Table 4: Grading of eyes before and after AAV.sFlt-1 and AAV.gfp injection and photoreceptor numbers/rows at 8 months post-injection

	Grades at time (months) post injection				Photoreceptor numbers	Rows of photoreceptors	Regression
Animal ID	[a]0	1	3	8			
243 L	1	0	0	0	68.8±14.1 [b]	4-8	Moderate
243 R	1	3	3	3	0	0	None
244 L	2	1	1	1	72.8±18.8 [b]	3-7	Moderate
244 R	1	3	2	1	5.8±3.1	0-1	None
247 L	2	2	0	0	80.8±31.0 [b]	3-8	Significant
247 R	1	1	1	1	28.3±33.3	0-4	None

249 L	3	1	1	1	0	0	Significant
249 R	3	3	3	4	7.2±13.1	0-1	None
250 L	3	1	1	1	65.1±24.4 [b]	3-8	Significant
250 R	3	3	3	3	0	0	None
251 L	3	2	1	1	0	0	Significant
251 R	3	3	3	4	0	0	None
253 L	3	2	1	1	73.8±20.39 [b]	5-7	Significant
253 R	3	2	3	2	20±30.3	0-2	Moderate
254 L	3	1	0	0	61.8±14.3 [b]	4-6	Significant
254 R	2	2	2	2	8.8±9.6	0-1	None
324 L	1	1	1	1	ND	ND	None
324 R	1	1	1	1	ND	ND	None
326 L	2	1	1	1	ND	ND	Moderate
326 R	2	2	2	2	ND	ND	None
327 L	2	2	0	0	ND	ND	Significant
327 R	2	3	2	2	ND	ND	None
329 L	3	2	2	2	ND	ND	Moderate
329 R	2	2	2	2	ND	ND	None
330 L	3	3	2	3	ND	ND	None
330 R	3	3	3	3	ND	ND	None

L= left eye injected with AAV.sFlt-1, R= right eye injected with AAV.GFP, ND= not done

[a]3 days prior to injection with AAV vectors.

[b]Statistically significant difference in photoreceptor numbers ($p<0.01$)

Example 11

Efficacy studies on a monkey model of laser-induced choroidal neovascularization

[00397] Five monkeys were injected in one eye with rAAV.sFlt-1 and in the other with rAAV.gfp. Subretinal injection was unsuccessful in the right eye of one of the monkeys; therefore this animal was not subjected to further evaluation. Subretinal injection of 40-100 µl of rAAV suspension lifted the retina, creating a bleb that housed the vector between the pigment epithelium and the photoreceptor layer in a localized manner. This bleb self-corrected within 24 to 48 hours. Except for a minor disturbance to the retinal pigment epithelium at the point of needle penetration, no other retinal abnormalities were observed for the duration of the follow-up (3 to 17 months post-injection). No other abnormalities or adverse events were observed; at no time was retinal detachment associated with the surgery.

[00398] To assess the long-term therapeutic efficacy of rAAVsFlt-1, the four injected monkeys were then subjected to intense laser photocoagulation 16 months after treatment with the vectors. Eight lesions were induced using laser in each eye, and the eyes then monitored for choroidal neovascularization at two and four weeks after laser treatment. After laser photocoagulation, only three of the four monkeys were analyzable, therefore, efficacy data was collected for three animals. None of the three monkey eyes treated with rAAVsFlt-1 developed choroidal neovascularization -related lesions and only weak fluorescein staining was observed, indicating minimum leakage / neovascularization. All contralateral eyes treated with control vector developed choroidal neovascularization-related lesions. Efficacy data for the three animals are presented in Table 5.

Table 5: Effect of subretinal administration of rAAV.sFlt-1 or control (rAAV.gfp) vector on laser-induced CNV in macaque monkeys

Monkey No.	Time of laser-induced CNV (months)*	CNV Lesions after Fluorescein Fundus Angiography†			
		Right Eye (rAAV.sFlt-1)		Left Eye (rAAV.gfp)	
		2 Weeks	4 Weeks	2 Weeks	4 Weeks
1	16	0 / 8	0 / 8	1 / 8	6 / 8
2	16	0 / 8	0 / 8	0 / 8	3 / 8
4	16	0 / 8	0 / 8	0 / 8	2 / 8

*CNV was induced at 16 months after subretinal injection of rAAVs.

†Number of macular lesions with neovascularization (fluorescein leakage) after laser photocoagulation.

The retinal function of the monkeys was assessed by electroretinography. Amplitudes and implicit times from the responses of the injected eye and uninjected contralateral eye were calculated and compared pre-injection and at different times following injection. The results showed that injection of rAAV.sFlt-1, the recombinant sFLT-1 protein or rAAV.gfp did not have any adverse effect on the retinal function of the monkeys.

Example 12

[00399] The standard of care in treating wet AMD involves frequent intraocular injection of recombinant anti-VEGF proteins every 4-8 weeks. A rAAV construct has been developed for a potent (Kd ~10 pM), naturally occurring anti-VEGF protein, soluble Fms-related tyrosine kinase-1 (sFlt-1), for the treatment of wet AMD. rAAV.sFlt-1 was produced in accordance with FDA and ICH guidelines at the UNC Vector Core Human

Application Laboratory. An eight patient controlled study on the safety and efficacy of rAAV.sFlt-1 was conducted. Eligibility, inclusion and exclusion criteria for the study were as follows:

[00400] Eligibility Criteria

Ages Eligible for Study:	65 Years and older
Genders Eligible for Study:	Both
Accepts Healthy Volunteers:	No

[00401] Inclusion Criteria:

- Age greater than or equal to 65 years;
- Subfoveal CNV secondary to AMD and with best corrected visual acuity of 20/80 – 20/400 or better in the other eye;
- Fluorescein angiogram of the study eye must show evidence of a leaking subfoveal choroidal neovascular lesion;
- Must be a candidate for anti-VEGF intravitreal injections;
- The entire dimension of the lesion must not exceed 12 Macular Photocoagulation Study disc areas;
- No previous retinal treatment of photodynamic therapy or laser;
- Able to provide informed consent;
- Participant has clinically acceptable laboratory and ECG at the time of enrolment; and
- Able to comply with protocol requirements, including follow-up visits.

[00402] Exclusion Criteria:

- Liver enzymes > 2 X upper limit of normal;
- Clinical evidence of active infection of any type, including adenovirus, hepatitis A, B, or C, or HIV virus;
- Any prior treatment for AMD in the study / control eye, excluding anti-VEGF injections;
- A tear in the retinal pigmented epithelium;
- Extensive submacular scar tissue;

- Significant retinal disease other than subfoveal CNV AMD, such as diabetic retinopathy or retinal vascular occlusion;
- Significant non-retinal disease such as ocular atrophy or cataracts;
- Known allergy to fluorescein;
- Current use of prednisolone, other anti-inflammatory steroids or immune suppression drugs. Non-steroidal drugs such as aspirin are allowed;
- Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study;
- Participants who have participated in another research study involving an investigational product in the past 12 weeks; and
- Penicillin sensitivity.

[00403] Administration procedure: The pharmaceutical composition containing rAAV.sFlt-1 was administered to study subjects in a setting appropriate for subretinal injection according to the following procedure:

1. The subject's periocular skin and eyelid margins and eye lashes were cleaned with 5% povidone iodine prior to draping;
2. A sterile whole body drape was placed followed by an additional eye drape.
3. Inserted eyelid speculum, ensuring that it is well positioned underneath the eyelids to direct the eyelashes away from the field and protected by eye drape.
4. Inserted 3 x 23G or 25G vitrectomy ports;
5. Connected saline infusion to 1st port;
6. Inserted fiber optic into 2nd port;
7. A 36G-41G subretinal cannula was connected to drug syringe via microconnector in the 3rd port;
8. Under microscopic control, 100 microlitres is injected under the retina;
9. Following injection, instruments and ports were withdrawn;
10. Chloramphenicol ointment was applied;
11. Atropine 1% drop from sterile single use container was instilled; and
12. An eye pad and eye shield were applied.

[00404] The results of the rAAV.sFlt-1 study are summarized herein.

The eight enrolled subjects (mean age 77 years) all had active subfoveal choroidal neovascularization, with visual acuity of 20/40 to 20/400, and had previously received between 1 and 25 intravitreal injections of ranibizumab. The patients were randomly distributed into three groups, a control group and two experimental groups. All patients received intravitreal injections of ranibizumab on day 1 and day 30 of the study. On day 7, 1 x 10^{10} vector genomes of rAAV.sFlt-1 in 100ul volume was administered via subretinal injection to the first experimental group and 1 x 10^{11} vector genomes of rAAV.sFlt-1 in 100ul volume was administered via subretinal injection to the second experimental group. In all six cases for patients in the experimental groups, the bleb of sub-retinal fluid resolved within 4 hours. After 24 hours, most of the air in the vitreous had absorbed and only the retinal injection site remained visible. One patient developed a minor hemorrhage associated with the procedure that did not affect vision. As expected following vitrectomy, there was a transient increase in neutrophil counts that returned to normal by 14 days post injection. Vector sequence was found in the tears of one subject at one day post injection that cleared by day 30. Other than this single occurrence, AAV2 was not detected in any of the subjects' blood, saliva or urine samples either by qPCR or ELISA to date. Background levels of the naturally occurring sFLT-1 protein showed a high baseline variation in the urine, serum, and saliva with no increase following treatment. sFLT-1 levels in the vitreous also varied among subjects (975-2085pg/ml). Blood biochemistry, complete blood count, and T-cell response, remained without any significant change compared to baseline. Subretinal injection of rAAV.sFlt-1 showed no clinically significant retinal toxicity as assessed by serial ophthalmic examinations over a two month period. No superficial, anterior segment or vitreous inflammatory signs were present in any of the subjects. There was no evidence of visual acuity loss, IOP elevation, retinal detachment, or any intraocular or systemic immune response in any of the patients. A summary of anti-VEGF treatments, both initial and rescue, are summarized for each patient in Table 6.

Table 6: Summary of Ranibizumab Injections by Patient

Subject	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180	Day 210	Day 224	Day 252	Day 280	Day 308	Day 336	Day 364
R1001	X	X	0	0	0	0	0	No visit	No visit	0	0	0	0	0
R1002	X	X	0	0	0	0	0	0	0	No visit	0	0	0	0
R1003 (control)	X	X	0	X	X	0	0	X	0	X	0	0	X	0
R1004	X	X	0	0	0	0	0	0	0	0	0	0	0	X
R1005	X	X	0	0	0	0	0	No visit	0	0	0	0	0	0
R1006	X	X	0	X	No visit	0	0	0	0	0	0	0	0	0
R1007 (control)	X	X	0	0	0	X	0	0	0	0	0	0	0	0
R1008	X	X	0	X	0	0	No visit	0	0	0	0	0	0	0

Note: Per protocol, injections at Day 0 and Day 30 were mandatory for all patients in the study\\

[00405] Notably, none of the patients in the experimental groups required rescue treatment at day 60 and most of the patients in the lower dose experimental group required 0 rescue treatments at day 90, day 120, day 150, day 180 or day 210 or day 270 or day 365 (1 year). The control patient required multiple rescue treatments. These results are unexpected and extend the promise of gene therapy for the large cohort of elderly patients suffering from wet AMD. Generally, patients treated with current anti-

VEGF therapy, such as intravitreal injections of a VEGF inhibitor protein or other anti-VEGF agent will require additional injections in 30, 60 or 90 days.

[00406] Maximum expression levels of sFLT-1 in a study subject or a patient are reached six to eight weeks after subretinal administration of rAAV.sFLT-1. During this so called "ramp-up" period, at least one, two or three intravitreal injections of an anti-VEGF agent are injected at 15 to 45 day intervals, and preferably about 30 day intervals, to prevent disease progression. It is preferred to administer the first intravitreal injection of an anti-VEGF agent between 1 to 30 days, and preferably between 5 to 10 days, prior to administration of rAAV.sFlt-1 to allow for absorption of the intravitreally injected anti-VEGF agent (Lucentis or Avastin or Eylea or other non sFLT agents). If this first intravitreal injection is administered less than 24 hours prior to subretinal administration of rAAV.sFLT, it may be washed out of the vitreous during the subretinal injection procedure leading to a sub-therapeutic anti-VEGF agent concentration and disease progression.

[00407] After the completion of the ramp period, patients who express sufficient sFLT-1 to treat or prevent progression of their AMD may not need additional intravitreal anti-VEGF injections although it is expect that they will remain under the care of a physician. Patients are monitored and treated on an as-needed basis based on objective criteria, such as an increased center point retinal thickness measurement with an optical coherence tomography.

[00408] In this study, patients in the control and both experimental groups were evaluated for signs of active choroidal neovascularization on an approximately monthly basis and retreated with intravitreal ranibizumab if any of the following criteria was met:

- >10 Early Treatment Diabetic Retinopathy Study (ETDRS) letter loss from subject's previous visit (attributable to retinal causes), OR a decrease of >5 ETDRS letters from previous visit in conjunction with patient perception of functional loss;
- Any increased, new, or persistent subsensory, sub-Retinal Pigment Epithelial (RPE), or intraretinal fluid on OCT;
- Signs of increased CNV leakage via FA.

Example 13

Optical Coherence Tomography (OCT)

[00409] Spectral Domain Optical Coherence Tomography (SD-OCT) was performed using approved equipment (Heidelberg Spectralis® SD-OCT) and standard techniques to monitor center point retinal thickness and fluid leakage in the retina of patients.

[00410] Optical Coherence Tomography (OCT) is a non-contact medical imaging technology similar to ultrasound and MRI. With OCT, reflected light is used to produce detailed cross-sectional and 3D images of the eye. The SPECTRALIS® SD-OCT simultaneously measures multiple wavelengths of reflected light across a spectrum, hence the name spectral domain. The increased speed and number of scans translates into higher resolution and a better chance of observing disease. In patients with wet AMD, the detection of new retinal fluid or a clinically significant increase in retinal thickness may be detected by SD-OCT. (Adhi et al., Curr Opin Ophthalmol. 2013 May; 24(3):213-21; Malamos et al., Invest Ophthalmol Vis Sci. 2009 Oct; 50(10):4926-33). Detection of these symptoms in a patient with AMD indicates disease progression that warrants treatment with an anti-VEGF therapy such as Lucentis or Eylea.

[00411] The retinal health and symptoms of AMD progression of each subject in the study were monitored via SD-OCT. At least 6 radial scans through the macula, each approximately 6mm in length, were taken; and OCT images/ scans were collected at each specified visit. The SD-OCT images were evaluated for the presence of intraretinal fluid by a masked reader and the central retinal thickness was measured using Heidelberg Heyex SD-OCT software. The central retinal thickness results for each visit for 8 patients are presented below in Table 7.

Table 7: Mean Change in Central Retinal Thickness from Baseline at Day 0 in microns by dosing group

Study Day	14	28	56	84	112	140	168	196	224	252	280	308	336	364
Control	-101	24	-194	-178	-176	-199	-131	-124	-186	-190	-198	-172	-157	-138
Low dose	-140	-115	-161	-189	-173	-163	-157	-147	-149	-155	-161	-144	-127	-134
High Dose	-254	-245	-266	-254	-245	-239	-235	-209	-219	-225	-215	-239	-246	-245

[00412] As shown in table 7, the mean central retinal thickness of the subjects in all dosing cohorts decreased after administration of the intravitreal injections of the anti-VEGF protein (Lucentis) at the beginning of the study as required by protocol. As expected, the central retinal thickness of the patients in the control group starts to increase and fluid can be seen on SD-OCT images within 30 – 90 days of the administration of the anti-VEGF protein. Unexpectedly, the central retinal thickness of the subjects in the low and high dosing groups is generally well controlled by rAAV.sFlt-1 and does not increase over time. New intraretinal fluid does not occur in the retinas of the low dose group subjects or the high dose group subjects. This is shown by OCT, for example, in Figure 24. At 12 months, the central retinal thickness of subjects treated with rAAV.sFlt-1 did not increase by more than 50 microns, or by more than 100 microns, or by more than 250 microns within 12 months of administration of a pharmaceutical composition comprising rAAV.sFlt-1. When compared against baseline, the central retinal thickness of human subjects treated with rAAV.sFlt-1 decreased by 50 microns or in some cases by 100 microns or in some cases, by 200 microns. This decrease was observed within 8 weeks of administering sFlt-1 and was maintained at 3 months, 6 months, 9 months and 12 months. This result is surprising and is unknown in in the clinical treatment of AMD and ocular neovascularization in human subjects. More generally, without additional administrations of an anti-VEGF protein or other VEGF inhibitor, intraretinal fluid and an increase in central retinal thickness will be observed with 30 days, 60 days, 90 days or 180 days of an initial anti-VEGF treatment.

Fluorescein Angiography (FA)

[00413] FA was performed using a standard technique. Transit images are taken of the study eye. Mid and late phase images are taken of the study and non-study eye; and FA is be obtained at each specified visit.

Biodistribution Studies

[00414] Dissemination of vector was investigated by polymerase chain reaction (PCR) amplification of vector genomes isolated from samples of tears, plasma, urine and saliva. Biodistribution of vector and sFLT-1 was investigated by ELISA for sFLT-1 and AAV2 capsids in plasma, tears and saliva.

Extraction of DNA

[00415] Samples (100-300 ul) were pipetted onto Sample Collection Cards (Qiagen, Valencia, CA) or sterile foam tip applicators. DNA was extracted from each sample as per

manufacturer's protocol. Purified DNA was dissolved in 50 ul of elution buffer. The amount of DNA present was determined by spectrophotometry.

Detection of rAAV.sFlt-1 by Real Time PCR

[00416] Genomic DNA samples (0.5-1 μg) were screened for the presence of the AAV.sFlt-1 vector using the TaqMan® Gene Expression Assays (Applied Biosystems, U.S.A.). The assay consists of a pair of unlabeled PCR primers which amplifies a fragment between the AAV2 and the sFLT-1 sequences, and a TaqMan® probe with a FAM™ or VIC® dye label and minor groove binder moiety on the 5' end, and non-fluorescent quencher dye on the 3' end. The cycling conditions were 1 hold for 2 minutes at 50°C and another hold at 95°C for 20 seconds, followed by 45 cycles of 95°C for 3 seconds and 60°C for 30 seconds.

[00417] Samples positive for the rAAV.sFlt-1 fragment were further tested and the gene copy number of rAAV.sFlt-1 present were quantified by real time polymerase chain reaction (PCR). Between 0.5-1.0 ug of extracted DNA were amplified in 20-ul reaction mixes containing Platinum SYBR Green qPCR Supermix-UDG (Invitrogen, Carlsbad, California, USA) and 0.5 uM of each primer using the IQ5 Bio-Rad real-time PCR system (Bio-Rad, Hercules, California, USA). A similar set of samples spiked with plasmid DNA containing the target sequence was set up in parallel as the spiked samples. The primer pair used (forward: CACTAGTCCAGTGTGGTGGA; reverse: AGCCAGGAGACAACCACTTC) was designed with the aid of Primer3 Output (Whitehead Institute, MA, USA) to amplify the region from the vector cDNA into the sFLT-1 gene using the Rotorgene (Corbett). The cycling conditions that were used were: 2 min 50.0 °C, 2 min 95.0 °C and 60 three-step cycles of 95.0 °C 20 s, 60.0 °C for 20 s and 72.0 °C for 20 s. A standard curve was generated in each run from 10-fold dilutions of plasmid DNA (pSSV.sFlt-1) which had the same target vector sequence. Each sample was analyzed in triplicate.

Quantifying sFlt-1 Protein Concentration by ELISA

[00418] The concentration of sFLT-1 present in the plasma, tears and saliva were measured quantitatively by ELISA using a Quantikine ELISA kit (R&D Systems, Minneapolis, MN) which was based on the sandwich immunoassay technique. The samples (100 ul) were added to the 96-well plate coated with a monoclonal antibody specific for VEGF R1/sFLT-1 and allowed to incubate for 2 hours. Any unbound sFLT-1 was removed by washing with a buffer. Following incubation with an enzyme-linked

polyclonal antibody specific for VEGF R1/sFLT-1, the excess of antibody-enzyme conjugate was washed off and the samples were then be incubated with a substrate solution. Enzyme-catalyzed chromogen formation was quantified by measuring the visible absorbance at 450 nm. The concentrations of sFLT-1 (in pg/ml) in the samples were calculated from the absorbance value using a calibration curve plotted with recombinant human sFLT-1.

Detection of AAV2 by ELISA

[00419] Presence of AAV2 capsid in the plasma, tears, urine and saliva was analyzed using the AAV2 Titration ELISA Kit (American Research Products, Inc., Belmont, Massachusetts, USA). This kit is based on a sandwich ELISA technique and uses a mouse monoclonal antibody specific for a conformational epitope on assembled AAV particles. This monoclonal antibody is coated onto microplate strips and is used to capture AAV particles from the specimen. Captured AAV particles were detected in two steps. First a biotin-conjugated monoclonal antibody to AAV was bound to the immune complex. In the second step streptavidin peroxidase conjugate reacts with the biotin molecules. Addition of substrate solution results in a color reaction which was proportional to specifically bound virus particles. The absorbance was measured photometrically at 450 nm. The kit control provided contains an AAV particle preparation of empty capsids and it allowed the quantitative determination of samples of an unknown particle titer. Samples (100 ul) were added to the plates and the assay was to be carried out according to the manufacturer's protocol.

Detection of Neutralizing AAV-2 Antibody

[00420] Plasma was assayed for the ability to block the transduction of HEK293 cells with AAV2.gfp. Patient's plasma was serially diluted in normal mouse serum in multi-well plates. AAV2.gfp was added to each well and plates were incubated at 37°C for 1 hour before addition to HEK293 cells in triplicate. The neutralizing antibody titer was expressed as the plasma dilution that resulted in 50% inhibition of transduction by AAV2-gfp. Maximum gfp activity was represented by vector diluted in normal mouse serum; maximum inhibition was represented by medium only in normal mouse serum. Baseline plasma from each subject was assayed alongside each post-op sample. Green cells from transduction of 293T cells with AAV2.gfp were counted in the test wells after 48 hours and compared with the number of green cells in the baseline serum sample.

Detection of Anti-AAV2 Antibodies

[00421] To detect plasma antibodies to AAV2 capsid, enhanced protein-binding ELISA plates were coated with 10^9 vg/ml of AAV2 (Vector Core Facility, North Carolina) at 4 °C overnight. The plates were be blocked at 37 °C for 2 hours and then are incubated at 4 °C overnight with serially diluted anti-AAV2 monoclonal antibody (Industries International, Concord, MA) or 1:50, 1:100, 1:200, or 1:400 dilutions of patient plasma. The plates were incubated with horse radish peroxidase (HRP)-conjugated anti-human Ig at 37 °C for 2 hours, then with tetramethyl benzidine (TMP) substrate and hydrogen peroxide (H2O2). The reaction was stopped by phosphoric acid (H3PO4) and read at 450 nm on a plate reader. The titer of anti-AAV2 antibodies were calculated based on the standard curve of the commercial antibody determined in parallel. Each value was determined in triplicate.

Geographic Atrophy

[00422] The human study subjects were examined for signs of geographic atrophy in their treated and untreated eyes according to standard techniques. Increases geographic atrophy was not observed in patients treated with rAAV.sFlt-1 at 3 months, 6 months, 9 months, or 12 months. It is hypothesized that the treatment may stop progression of geographic atrophy in a treated eye for up to 15 months, 18 months, 24 months, 36 months, 5 years and 10 years.

Example 14

[00423] To further test the safety and efficacy of rAAV.sFlt-1 for the treatment of wet AMD and choroidal neovascularization, forty (40) additional subjects were enrolled in a controlled clinical study. As in Example 12, rAAV.sFlt-1 was produced in accordance with FDA and ICH guidelines at the UNC Vector Core Human Application Laboratory. Eligibility, inclusion and exclusion criteria for the study were as follows:

[00424] Eligibility:

Ages Eligible for Study: 55 Years and older

Genders Eligible for Study: Both

Accepts Healthy Volunteers: No

[00425] Inclusion Criteria:

- Age greater than or equal to 55 years;

- Subfoveal CNV secondary to AMD and with best corrected visual acuity in the study eye of 20/30 – 20/400 and 20/200 or better in the other eye;
- Fluorescein angiogram of the study eye must show evidence of a leaking subfoveal choroidal neovascular lesion; or choroidal neovascularization currently under active management with anti-VEGF therapy;
- Must be a candidate for anti-VEGF intravitreal injections;
- The entire dimension of the lesion must not exceed 12 Macular Photocoagulation Study disc areas;
- No previous retinal treatment of photodynamic therapy or laser;
- Able to provide informed consent;
- Participant has clinically acceptable laboratory parameters and ECG at the time of enrollment; and
- Able to comply with protocol requirements, including follow-up visits.

[00426] Exclusion Criteria:

- Liver enzymes > 2 X upper limit of normal;
- Clinical evidence of active infection of any type, including adenovirus, hepatitis A, B, or C, or HIV virus; or documented history of hepatitis B or hepatitis C;
- Any prior treatment for AMD in the study / control eye, excluding anti-VEGF injections;
- A tear in the retinal pigmented epithelium;
- Extensive sub-fovial scarring, extensive geographic atrophy, or thick subretinal blood in the study eye as determined by the investigator;
- Significant retinal disease other than subfoveal CNV AMD, such as diabetic retinopathy or retinal vascular occlusion, that could compromise vision in the study eye;
- Significant non-retinal disease such as ocular atrophy or significant cataract in the study eye, including central corneal scarring that affects visual acuity, glaucoma with field defects, or any measurable uveitis;

- Known allergy to fluorescein;
- Current use of prednisolone, other anti-inflammatory steroids or immune suppression drugs. Inhaled steroids and non-steroidal drugs such as aspirin are allowed;
- Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study;
- Participants who have participated in another research study involving an investigational product in the past 12 weeks; and
- Penicillin sensitivity confirmed by participant medical records.

[00427] Initial enrolled subjects had active subfoveal choroidal neovascularization, with visual acuity in the study eye of 20/30 to 20/400, and had previously received between 0 and 25 intravitreal injections of ranibizumab. The patients were randomly distributed into a control group or an experimental group until a total of 14 patients control patients and 26 experiments patients were enrolled. All patients received intravitreal injections of ranibizumab on day 1 and day 30 of the study. On day 7, 1 x 10^{11} vector genomes of rAAV.sFlt-1 in 100 ul volume was administered via subretinal injection to the experimental group.

[00428] As in the study in Example 12, maximum expression levels of sFLT-1 in a study subject or a patient were reached six to eight weeks after subretinal administration of rAAV.sFLT-1. During this so called "ramp-up" period, at least one, two or three intravitreal injections of an anti-VEGF agent were injected at 15 to 45 day intervals, and preferably about 30 day intervals, to prevent disease progression. It is preferred to administer the first intravitreal injection of an anti-VEGF agent between 1 to 30 days, and preferably between 5 to 10 days, prior to administration of rAAV.sFLT-1 to allow for absorption of the intravitreally injected anti-VEGF agent (Lucentis or Avastin or Eylea or other non sFLT agents). If this first intravitreal injection is administered less than 24 hours prior to subretinal administration of rAAV.sFLT, it may be washed out of the vitreous during the subretinal injection procedure leading to a sub-therapeutic anti-VEGF agent concentration and disease progression.

[00429] After the completion of the ramp period, patients who expressed sufficient sFLT-1 to treat or prevent progression of their AMD or other symptoms of choroidal neovascularization did not need additional intravitreal anti-VEGF injections although it is expected that they will remain under the care of a physician.

[00430] In this study recited in this example, patients in the control and experimental groups were evaluated for signs of active choroidal neovascularization on an approximately monthly basis and retreated with intravitreal ranibizumab if any of the following criteria was met:

- >10 Early Treatment Diabetic Retinopathy Study (ETDRS) letter loss from subject's previous visit (attributable to retinal causes), OR a decrease of >5 ETDRS letters from previous visit in conjunction with patient perception of functional loss;
- Any increased, new, or persistent subsensory, sub-Retinal Pigment Epithelial (RPE), or intraretinal fluid on OCT;
- Signs of increased CNV leakage via FA.

Example 15

[00431] To test the safety and efficacy of rAAV.sFlt-1 for the prevention or prophylaxis of the ocular neovascular disease Age Related Macular degeneration (AMD), an additional controlled clinical study with forty (150) patients is conducted. rAAV(bv).sFlt-1 is produced in accordance with FDA and ICH guidelines at Lonza Houston, Inc. (Houston, Texas). Eligibility, inclusion and exclusion criteria for the study were as follows:

[00432] Eligibility:

- Ages Eligible for Study: 50 Years and older
- Genders Eligible for Study: Both
- Accepts Healthy Volunteers: Yes

[00433] Inclusion Criteria:

- Patients with nonexudative AMD (either categories 2, 3 or 4 according to the AREDS criteria; in group 4 the eyes with no-advanced AMD will be included); Patients with AMD classified as either "wet" or "dry" are included;
- Age between 50 and 90 years;

- Able to understand and comply with the requirements of the trial;
- Visual acuity > 0.4;

[00434] Exclusion Criteria:

- Currently enrolled in an ophthalmic clinical trial;
- Eyes with concomitant macular or choroidal disorders other than AMD and with indefinite signs of AMD;
- Eyes with a diagnosis of exudative AMD with active subretinal neovascularization (SRNV) or CNV lesions requiring laser photocoagulation in the study eye;
- Subjects with significant ocular lens opacities causing vision decrease;
- Subjects with amblyopia;
- Subjects with optic nerve disease (neuropathy, atrophy, papilledema), unstable glaucoma as defined by intraocular pressures greater than 25 mm Hg, 3 or more glaucoma medications, C/D of 0.8 or greater and visual fields consistent with glaucoma; history of retina-vitreous surgery, degenerative myopia, active posterior intraocular inflammatory disease, chronic use of topical ocular steroid medications, vasoproliferative retinopathies (other than AMD), rhegmatogenous retinal detachment, and inherited macular dystrophies;
- Subjects with demand type pacemakers or epilepsy;
- Subjects with uncontrolled hypertension (defined as diastolic of 90 or greater and systolic of 150 or greater);
- Subjects with recent history (within the previous year) of cerebral vascular disease;
- manifested with transient ischemic attacks (TIA's) or cerebral vascular accidents (CVA's);
- Subjects with a history of AIDS;
- Subjects who have had intraocular surgery in trial eye within 3 months prior to enrolling in the trial;
- Patients who are unwilling to adhere to visit examination schedules;

[00435] Primary Outcome Measures:

MPOD and multifocal electroretinograms [Time Frame: 1 year] [Designated as safety issue: Yes]

[00436] Secondary Outcome Measures:

The safety and efficacy of rAAV(bv).sFlt-1 in reducing the risk of the development of advanced AMD. [Time Frame: 1 year] [Designated as safety issue: Yes]

Table 9: Experimental Design Arms

Arms	Assigned Interventions
Active Comparator: Group I 1 x 10^{10} vector genomes of rAAV(bv).sFlt-1 in 100ul volume is administered via subretinal injection to the experimental group within 30-90 day intervals for 36 months	Drug: of rAAV(bv).sFlt-1 1 x 10^{10} vector genomes of rAAV.sFlt-1 in 100ul volume is administered via subretinal injection to the experimental group.
Active Comparator: Group II 1 x 10^{11} vector genomes of rAAV(bv).sFlt-1 in 100ul volume is administered via subretinal injection to the experimental group within 180-365 day intervals for 36 months	Drug: of rAAV(bv).sFlt-1 1 x 10^{11} vector genomes of rAAV.sFlt-1 in 100ul volume is administered via subretinal injection to the experimental group.
Placebo Comparator: Group Placebo Drug Placebo: Saline solution	Drug Placebo: Saline solution Drug Placebo: Saline solution, until one year. Patients on placebo showing early stages of AMD may receive rAAV(bv).sFlt-1
Active Comparator: Ranibizumab 0.3 mg Patients receive ranibizumab 0.3 mg monthly administered intravitreally for 36 months.	Drug: Ranibizumab Sterile solution for intravitreal injection. Other Name: Lucentis

Example 16

[00437] To test the safety and efficacy of rAAV.sFlt-1 for the treatment of the ocular neovascular disease Diabetic Macular Edema (DME), an additional controlled clinical study with forty (40) patients is conducted. rAAV(bv).sFlt-1 is produced in accordance with FDA and ICH guidelines at Lonza Houston, Inc. (Houston, Texas). Eligibility, inclusion and exclusion criteria for the study were as follows:

[00438] Eligibility:

- Ages Eligible for Study: 18 Years and older
- Genders Eligible for Study: Both
- Accepts Healthy Volunteers: No

[00439] General Inclusion Criteria:

- Subjects are eligible if the following criteria are met:
- Willingness to provide written informed consent and, at U.S. sites, Health Insurance Portability and Accountability Act (HIPAA) authorization, and in other countries, as applicable according to national laws.
- Diabetes mellitus (Type 1 or 2).
- Retinal thickening secondary to diabetes mellitus (DME) involving the center of the fovea with central macular thickness ≥ 275 μm in the center subfield as assessed on optical coherence tomography (OCT).
- Best corrected visual acuity (BCVA) score in the study eye of 20/40 to 20/320 approximate Snellen equivalent using the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol at an initial testing distance of 4 meters.
- Decrease in vision determined to be primarily the result of DME and not to other causes.
- Ability (in the opinion of the investigator) and willingness to return for all scheduled visits and assessments.

[00440] Exclusion Criteria:

- History of vitreoretinal surgery in the study eye.
- Panretinal photocoagulation (PRP) or macular laser photocoagulation in the study eye within 3 months of screening.
- Proliferative diabetic retinopathy (PDR) in the study eye, with the exception of inactive, regressed PDR.
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in the study eye.
- Vitreomacular traction or epiretinal membrane in the study eye.
- Ocular inflammation (including trace or above) in the study eye.
- History of idiopathic or autoimmune uveitis in either eye.
- Structural damage to the center of the macula in the study eye that is likely to preclude improvement in VA following the resolution of macular edema, including atrophy of

the retinal pigment epithelium (RPE), subretinal fibrosis, or organized hard-exudate plaque.

- Ocular disorders in the study eye that may confound interpretation of study results, including retinal vascular occlusion, retinal detachment, macular hole, or choroidal neovascularization (CNV) of any cause (eg, age-related macular degeneration (AMD), ocular histoplasmosis, or pathologic myopia).
- Cataract surgery in the study eye within 3 months, yttrium-aluminum-garnet (YAG) laser capsulotomy within the past 2 months, or any other intraocular surgery within the 90 days preceding Day 0.
- Uncontrolled glaucoma or previous filtration surgery in the study eye.
- Uncontrolled blood pressure.
- History of cerebral vascular accident or myocardial infarction within 3 months prior to Day 0.
- Uncontrolled diabetes mellitus.
- Renal failure requiring dialysis or renal transplant.
- History of other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use an investigational drug, might affect interpretation of the results of the study, or renders the subject at high risk from treatment complications.

[00441] Primary Outcome Measures:

- Percentage of Patients Who Gain ≥ 15 Letters in Their Best Corrected Visual Acuity (BCVA) Score From Baseline at Month 12 [Time Frame: Baseline to Month 12] [Designated as safety issue: No]

[00442] Secondary Outcome Measures:

- Mean Change From Baseline in Best Corrected Visual Acuity (BCVA) Score at Months 12, 24 and 36
- Percentage of Patients With a Visual Acuity (VA) Snellen Equivalent of 20/40 or Better at Months 12, 24 and 36.
- Mean Change From Baseline in Central Foveal Thickness as measured by SD-OCT at Months 12, 24 and 36.
- Reduction in Frequency of concomitant anti-VEGF treatment (e.g. Lucentis, Avastin, Macugen or Eyelea) [Designated as safety issue: No]

Table 10: Experimental Design Arms for DME Study

Arms	Assigned Interventions
Experimental: I Low Dose 1 x 10^{10} vector genomes of rAAV(bv).sFlt-1 in 100ul volume was administered via subretinal injection to the experimental group. Follow-up phase: Participants on rAAV.sFlt-1 are monitored monthly and receive rescue treatments with intravitreal anti-VEGF therapy if they meet the study criteria for retreatment.	Drug: rAAV.sFlt-1 (AVA-01) 1 x 10^{10} vector genomes of rAAV.sFlt-1 in 100ul volume was administered via subretinal injection to the experimental group.
Experimental: II High Dose 1 x 10^{11} vector genomes of rAAV.sFlt-1 in 100ul volume was administered via subretinal injection to the experimental group. Follow-up phase: Participants on rAAV.sFlt-1 are monitored monthly and receive rescue treatments with intravitreal anti-VEGF therapy if they meet the study criteria for retreatment.	Drug: rAAV.sFlt-1 (AVA-01) 1 x 10^{11} vector genomes of rAAV.sFlt-1 in 100ul volume was administered via subretinal injection to the experimental group.
Active Comparator: Ranibizumab injection 0.3 mg. Participants receive two initial injections of Ranibizumab at Day 0 and Day 30. Follow-up phase: Participants are monitored monthly and receive rescue treatments with Ranibizumab if they meet the study criteria for retreatment.	Drug: Ranibizumab Sterile solution for intravitreal injection. Other Name: Lucentis

[00443] Initial enrolled subjects have DME, with visual acuity in the study eye of 20/40 to 20/320, and will have previously received between 0 and 25 intravitreal injections of ranibizumab or aflibercept. The patients are randomly distributed into a control group or two experimental groups until a total of 14 patients control patients and 13 low dose experimental patients and 13 high dose experimental patients are enrolled. All patients received intravitreal injections of ranibizumab on day 1 and day 30 of the study. On day

7, 1 x 10^{10} or 1 x 10^{11} vector genomes of rAAV(bv).sFlt-1 in 100 ul volume are administered via subretinal injection to the experimental groups.

[00444] As in the study in Example 12, maximum expression levels of sFLT-1 in a study subject or a patient are reached are six to eight weeks after subretinal administration of rAAV(bv).sFLT-1. During this so called "ramp-up" period, at least one, two or three intravitreal injections of an anti-VEGF agent are injected at 15 to 45 day intervals, and preferably about 30 day intervals, to prevent disease progression. It is preferred to administer the first intravitreal injection of an anti-VEGF agent between 1 to 30 days, and preferably between 5 to 10 days, prior to administration of rAAV(bv).sFLT-1 to allow for absorption of the intravitreally injected anti-VEGF agent (Lucentis or Avastin or Eylea or other non sFLT agents). If this first intravitreal injection is administered less than 24 hours prior to subretinal administration of rAAV(bv).sFLT, it may be washed out of the vitreous during the subretinal injection procedure leading to a sub-therapeutic anti-VEGF agent concentration and disease progression.

[00445] After the completion of the ramp period, patients who express sufficient sFLT-1 to treat or prevent progression of their DME may not need additional intravitreal anti-VEGF injections although it is expect that they will remain under the care of a physician.

[00446] In this study recited in this example, patients in the control and experimental groups are evaluated for signs of active or new DME and neovascularization on an approximately monthly basis and are retreated with intravitreal ranibizumab if any of the following criteria was met:

- >10 Early Treatment Diabetic Retinopathy Study (ETDRS) letter loss from subject's previous visit (attributable to retinal causes), OR a decrease of >5 ETDRS letters from previous visit in conjunction with patient perception of functional loss;
- Any increased, new, or persistent subsensory, sub-Retinal Pigment Epithelial (RPE), or intraretinal fluid on OCT;
- Signs of increased CNV leakage via FA.

Example 17

[00447] To test the safety and efficacy of rAAV.sFlt-1 for the treatment of the ocular neovascular disease Retinal Vein Occlusion (RVO), an additional controlled clinical study with forty (40) patients is conducted. The clinical study is performed with patients of 2 cohorts, 1 cohort including patients with Central Retinal Vein Occlusion (CRVO)

and 1 cohort including Branched Retinal Vein Occlusion (BRVO). As in Example 15, rAAV(bv).sFlt-1 is produced in accordance with FDA and ICH guidelines at Lonza Houston, Inc. (Houston, Texas). Eligibility, inclusion and exclusion criteria for the study were as follows:

[00448] Inclusion Criteria:

- Center-involved macular edema secondary to central retinal vein occlusion (CRVO) or Branch-involved macular edema secondary to BRVO for no longer than 9 months with mean central subfield thickness ≥ 250 μm on optical coherence tomography (OCT);
- Adults ≥ 18 years;
- Early treatment diabetic retinopathy study (ETDRS) best corrected visual acuity (BCVA) of 20/40 to 20/320 (73 to 24 letters) in the study eye;

[00449] Exclusion Criteria:

- Any prior treatment with anti-VEGF agents in the study eye (Pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, etc.) or previous administration of systemic anti-angiogenic medications;
- Prior panretinal laser photocoagulation or macular laser photocoagulation in the study eye
- CRVO disease duration > 9 months from date of diagnosis; BRVO disease duration > 9 months from date of diagnosis;
- Previous use of intraocular corticosteroids in the study eye or use of periocular corticosteroids in the study eye within the 3 months prior to Day 1;
- Iris neovascularization, vitreous hemorrhage, traction retinal detachment, or preretinal fibrosis involving the macula in either the study eye or fellow eye;

[0001] Primary Outcome Measures:

- Mean Change From Baseline in Best Corrected Visual Acuity (BCVA) Score at 6 Months. [Time Frame: Baseline and 6 months] [Designated as safety issue: No] .
- Defined study baseline range of Early Treatment Diabetic Retinopathy Study (ETDRS) Best Corrected Visual Acuity (BCVA) letter score of 73 to 24 (= Acuity of 20/40 to 20/320) in the study eye; a higher score represents better functioning. Nominator = (Number of participants who maintained vision * 100); Denominator = Number of participants analyzed.

[0002] Secondary Outcome Measures:

- Percentage of Participants Who Gained ≥ 15 Letters in BCVA Score at Month 6 Compared With Baseline.
- Mean Change From Baseline in Central Retinal Thickness (CRT) at 6 months [Time Frame: Baseline and 6 months] [Designated as safety issue: No]
- Reduction in frequency of concomitant anti-VEGF treatment ((e.g. Lucentis, Avastin, Macugen or Eyelea) [Designated as safety issue: No]

Table 11: Experimental Design Arms for BRVO/CRVO Study

Arms	Assigned Interventions
Experimental: 1 x 10^{10} vector genomes of rAAV.sFlt-1 in 100ul volume is administered via subretinal injection to the experimental group, on Day 7. Follow-up phase: Participants on rAAV.sFlt-1 are monitored monthly and receive rescue treatments with intravitreal anti-VEGF therapy if they meet the study criteria for retreatment.	Biological: 1 x 10^{10} vector genomes of rAAV.sFlt-1. Subretinal injection. Drug: Ranibizumab injection 0.3 mg if meet reinjection criteria.
Experimental: 1 x 10^{11} vector genomes of rAAV.sFlt-1 in 100ul volume is administered via subretinal injection to the experimental group, on Day 7. Follow-up phase: Participants on rAAV.sFlt-1 are monitored monthly and receive rescue treatments with intravitreal anti-VEGF therapy if they meet the study criteria for retreatment.	Biological: 1 x 10^{11} vector genomes of rAAV.sFlt-1. Subretinal injection. Drug: Ranibizumab injection 0.3 mg if meet reinjection criteria.
Active Comparator: Ranibizumab injection 0.3 mg. Participants receive two initial injections of Ranibizumab at Day 0 and Day 30. Follow-up phase: Participants are monitored monthly and receive rescue treatments with Ranibizumab if they meet the study criteria for retreatment.	Drug: Ranibizumab injection 0.3 mg Ranibizumab injection 0.3 mg in a single-dose regimen given at Day 0 and Day 30. Other Name: Lucentis

[00450] Initial enrolled subjects have CRVO or BRVO, with visual acuity in the study eye of 20/40 to 20/320, and will have previously received between 0 and 25 intravitreal injections of ranibizumab or aflibercept. The patients are randomly distributed into a control group or two experimental groups until a total of 14 patients control patients and 13 low dose experimental patients and 13 high dose experimental patients are enrolled. All patients received intravitreal injections of ranibizumab on day 1 and day 30 of the study. On day 7, 1 x 10^{10} or 1 x 10^{11} vector genomes of rAAV(bv).sFlt-1 in 100 ul volume are administered via subretinal injection to the experimental groups.

[00451] As in the study in Example 14, maximum expression levels of sFLT-1 in a study subject or a patient are reached are six to eight weeks after subretinal administration of rAAV(bv).sFLT-1. After the completion of the ramp period, as described in Example 14, patients who express sufficient sFLT-1 to treat or prevent progression of their BRVO or CRVO may not need additional intravitreal anti-VEGF injections although it is expect that they will remain under the care of a physician.

[00452] In this study recited in this example, patients in the control and experimental groups are evaluated for signs of active or new retinal vein occlusion and neovascularization on an approximately monthly basis and are retreated with intravitreal ranibizumab if any of the following criteria was met:

- >10 Early Treatment Diabetic Retinopathy Study (ETDRS) letter loss from subject's previous visit (attributable to retinal causes), OR a decrease of >5 ETDRS letters from previous visit in conjunction with patient perception of functional loss;
- Any increased, new, or persistent subsensory, sub-Retinal Pigment Epithelial (RPE), or intraretinal fluid on OCT;
- Signs of increased CNV leakage via FA.

CLAIMS

WHAT IS CLAIMED IS:

1. A nucleic acid comprising a sequence encoding a VEGF inhibitor for use in treatment or prophylaxis of ocular neovascularization in a human subject; 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.
2. The nucleic acid for use according to claim 1, wherein said VEGF inhibitor is an anti-VEGF antibody or functional fragment thereof.
3. The nucleic acid for use according to claim 1, wherein said VEGF inhibitor is soluble receptor, fusion protein, or fragment thereof.
4. A nucleic acid comprising a sequence encoding sFLT-1 for use in treatment or prophylaxis of ocular neovascularization in a human subject; 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.
5. The nucleic acid for use according to claim 4, 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.
6. The nucleic acid for use according to claim 4, 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.
7. The nucleic acid for use according to any preceding claim, 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.

8. The nucleic acid for use according to claim 7, wherein the recombinant virus is an AAV selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, and hybrids thereof.
9. The nucleic acid for use according to any of preceding claim, 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.
10. The nucleic acid for use according to any preceding claim, wherein the nucleic acid is a plasmid or is delivered by a viral vector.
11. The nucleic acid for use according to any preceding claim, 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, bevacizumab or aflibercept.
12. The nucleic acid for use according to any preceding claim, said use comprising administering said pharmaceutical composition to a human subject at least 55 years old.
13. The nucleic acid for use according to any preceding claim, said use comprising administering said pharmaceutical composition outside the fovea.
14. The nucleic acid for use according to any preceding claim, wherein the best corrected visual acuity (BCVA) of the human subject in need of treatment, as measured by ETDRS (Early Treatment Diabetic Retinopathy Study) letters, improves by at least 1, 2, 3, 4 or 5 lines following the administering of an effective amount of the pharmaceutical composition.
15. The nucleic acid for use according to any preceding claim, wherein the administering of the pharmaceutical composition reduces the frequency of administration of other anti-VEGF therapy.
16. The nucleic acid of claim 15, wherein the administering of other anti-VEGF therapy is less than once per 3 months or less than once per 6 months or less than once per year.

17. The nucleic acid for use according to any preceding claim, wherein the administering of the pharmaceutical composition is performed at a frequency less than 3 times a year in the human.
18. A unit dose of a pharmaceutical composition comprising a recombinant virus, wherein the unit comprises at least $1x10^{10}$ to $3x10^{12}$ vector genomes, and wherein the recombinant viruses comprise a nucleic acid encoding the sFLT-1 operatively linked to a promoter and wherein the unit dose is capable of elevating levels of sFLT-1 protein in the vitreous of a human subject after at least 7 days, after administering to the human subject.
19. A pharmaceutical composition comprising recombinant viruses or plasmids wherein each recombinant virus or plasmid comprises a nucleic acid, wherein the nucleic acid comprises an sFLT-1 transgene sequence operatively linked to a promoter sequence, and wherein the pharmaceutical composition is capable of increasing the level of sFLT-1 protein in the vitreous of a human subject after at least 7 days after administering to the human subject.
20. The pharmaceutical composition of claim 19, wherein the promoter sequence and the sFLT-1 transgene sequence are separated by a sequence greater than 300 base pairs.
21. The pharmaceutical composition of claim 19, wherein the promoter sequence and the sFLT-1 transgene sequence are separated by a UTR sequence
22. A pharmaceutical composition comprising nucleic acid elements in the following order:
    a. 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 and SEQ ID No. 32;
    b. 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 SEQ ID No. 108 or SEQ ID No. 122;
    c. 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, SEQ ID No. 119 or SEQ ID No.120;

d. 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
e. 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.

23. A pharmaceutical composition comprising nucleic acid elements in the following order:
    a. an ITR sequence selected from the group consisting of SEQ ID No. 56, SEQ ID No. 57, SEQ ID No. 58, SEQ ID No. 59;
    b. a linker sequence selected from the group consisting of SEQ ID No. 60, SEQ ID No. 63, SEQ ID No. 68, SEQ ID No. 72, SEQ ID No. 76, SEQ ID No. 77, and SEQ ID No. 84;
    c. 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 and 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. 40, 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;
    d. a linker sequence selected from the group consisting of SEQ ID No. 60, SEQ ID No. 64, SEQ ID No. 70, SEQ ID No. 71, SEQ ID No. 73, SEQ ID No. 74, SEQ ID No. 87;
    e. 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, SEQ ID No. 119 and SEQ ID No. 120;
    f. a linker sequence selected from the group consisting of SEQ ID No. 60, SEQ ID No. 64, SEQ ID No. 65, SEQ ID No. 75, SEQ ID No. 79, SEQ ID No. 81, SEQ ID No. 82, and SEQ ID No. 89;
    g. 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

h. 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. 107, SEQ ID No. 108 and SEQ ID No. 122;

i. 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;

j. a linker sequence selected from the group consisting of SEQ ID No. 60, SEQ ID No. 62, SEQ ID No. 66, SEQ ID No. 67, SEQ ID No. 69, SEQ ID No. 83, SEQ ID No. 84, SEQ ID No. 85, SEQ ID No. 86;

k. 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;

l. a linker sequence selected from the group consisting of SEQ ID No. 60, SEQ ID No. 61, SEQ ID No. 80, SEQ ID No. 87, SEQ ID No. 88, SEQ ID No. 89, SEQ ID No. 90; and

m. an ITR sequence selected from the group consisting of SEQ ID No. 56, SEQ ID No. 57, SEQ ID No. 58, and SEQ ID No. 59.

24. A method of generating a recombinant virus in a cell, the method comprising:

a. introducing into a cell, a nucleic acid comprising at least 1 promoter sequence operatively linked to a sFLT-1 transgene sequence, an ITR sequence, and a UTR sequence; and

b. purifying the recombinant virus.

25. 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.

26. 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.

27. The method of claim 26, wherein said VEGF inhibitor is an anti-VEGF antibody against VEGF or a functional fragment thereof.
28. The method of claim 26, wherein said VEGF inhibitor is a soluble receptor, fusion protein, or a functional fragment thereof.
29. 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.
30. The method of claim 29, wherein the human subject 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.
31. The method of claim 29, wherein 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.
32. The method of claim 29, wherein the pharmaceutical composition 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.
33. The method of claim 29, 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.
34. The method of claim 29, wherein the sFLT-1 nucleic acid encodes at least 1 dimerization domain.
35. The method of claim 29, wherein the nucleic acid does not contain a prokaryotic regulatory sequence.
36. The method of claim 29, wherein the nucleic acid contains a prokaryotic regulatory sequence.

37. The method of claim 29, wherein the nucleic acid is a plasmid or is delivered by a virus.
38. The method of claim 29, further comprising administration of one or more treatments of a VEGF inhibitor to the human subject.
39. The method of claim 38, wherein the VEGF inhibitor is administered within 30, 90, or 180 days of administration of the pharmaceutical composition.
40. The method of claim 38, wherein the pharmaceutical composition and the VEGF inhibitor are administered at least 24 hours apart.
41. The method of claim 29, wherein the pharmaceutical composition is administered to a human subject at least 55 years old.
42. The method of claim 29, wherein the administering is performed outside the fovea.
43. The method of claim 29, wherein the best corrected visual acuity (BCVA) of the human subject, as measured by ETDRS (Early Treatment Diabetic Retinopathy Study) letters, improves by at least 1, 2, 3, 4 or 5 lines following the administering of the pharmaceutical composition.
44. The method of claim 29, wherein the best corrected visual acuity (BCVA), as measured by ETDRS (Early Treatment Diabetic Retinopathy Study), decreases by fewer than 15 letters following the administering of the pharmaceutical composition.
45. The method of claim 29, wherein administering the pharmaceutical composition is performed 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.
46. The method of claim 29, wherein a reduction in neovascularization as observed by a Fluorscein Angiography (FA) follows the administering of the pharmaceutical composition.
47. The method of claim 29, wherein no superficial, anterior segment or vitreous inflammatory signs are present in the human subject at least 1 week after injection.
48. The method of claim 29, wherein the human subject does not require rescue treatment for at least 30, 60, 90, 120, 180, 270 or 365 days after the administering of the pharmaceutical composition.

49. The method of claim 32, wherein no increased anti-AAV cytotoxic T cell response is measured following the administering step.
50. The method of claim 32, wherein no virus is detected in the human subject's blood, saliva or urine samples, 3, 7, 14, 21 or 30 days after administering the pharmaceutical composition.
51. The method of claim 29 wherein the sFLT-1 protein level in the vitreous of the human subject is 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.
52. The method of claim 29, wherein the human subject has received one or more treatments with a VEGF inhibitor prior to the administering of the pharmaceutical composition.
53. The method of claim 29, wherein the human subject has not previously received treatment with a VEGF inhibitor before the administering of the pharmaceutical composition.
54. The method of claim 29, wherein the administering of the pharmaceutical composition is performed at a frequency less than 3 times a year in the human subject.
55. The method of claim 29, wherein the administering of the pharmaceutical composition reduces the frequency of administration of additional VEGF inhibitor treatments in the human subject.
56. The method of claim 29, wherein the concentration of sFLT-1 protein in the vitreous of the human subject is elevated when measured at 7, 14, 21, 30, 60, 90, 120, 150, 180, 270 or 365 days after the administering of the pharmaceutical composition.
57. The method of claim 29, wherein the human subject has the vitreous gel removed prior to or within one week of the administration of said agent.
58. The method of claim 29, wherein said pharmacological agent is administered using a vitrectomy system that is smaller than 20 gauge.
59. The method of claim 29, wherein said pharmacological agent is administered using a cannula tip that is smaller than 39 gauge.

60. The method of claim 29, wherein the central retinal thickness of said subject does not increase by more than 50 microns, 100 microns, or 250 microns within 12 months following treatment with said pharmacological agent.
61. The method of claim 29, wherein geographic atrophy does not progress in the diseased eye of the human subject.
62. The method of claim 29, wherein the human subject has sensitivity or allergy to penicillin.
63. The method of claim 29, wherein the human subject has no increase in systemic levels of VEGF inhibitor.
64. A pharmaceutical composition comprising recombinant viruses or plasmids comprising a nucleic acid comprising at least 1 promoter sequence operatively linked to an sFLT-1 transgene sequence, wherein the promoter sequence and the sFLT-1 transgene sequence are separated by a sequence greater than 300 base pairs.
65. A pharmaceutical composition comprising recombinant viruses or plasmids comprising a nucleic acid comprising at least 1 promoter sequence operatively linked to an sFLT-1 transgene sequence, wherein the promoter sequence and the sFLT-1 transgene sequence are separated by a UTR sequence.
66. The pharmaceutical composition of claim 65, wherein the UTR sequence comprises at least 10 base pairs.
67. The pharmaceutical composition of claim 66, wherein the nucleic acid comprises at least 3 linker sequences each comprising at least 50 base pairs.
68. A unit dose of a pharmaceutical composition comprising recombinant viruses of $1x10^{10}$ to $3x10^{12}$ vector genomes, wherein the recombinant viruses comprise a nucleic acid encoding sFLT-1 operatively linked to a promoter.
69. A method of generating a recombinant virus in a cell, the method comprising:
    a. 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
    b. purifying the recombinant virus.
70. The method of claim 69, wherein said nucleic acid sequence does not contain a beta-lactam antibiotic resistance sequence.

71. The method of claim 69, wherein 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 multiplicity of infection of $1x10^4$-$1x10^6$.
72. The method of claim 69, wherein the recombinant virus inhibits proliferation of human umbilical vascular endothelial (HUVEC) cells.
73. A cell for generating a 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.
74. The method of claim 29, wherein 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.
75. The method of claim 29, wherein the human subject experiences no visual acuity loss, IOP elevation, retinal detachment, intraocular or systemic immune response after administering the pharmaceutical composition.
76. The method of claim 29, wherein the human subject is resistant to treatment with VEGF inhibitors.
77. The method of claim 29, wherein said pharmacological agent is administered using a vitrectomy system that does not require sutures.
78. The method of claim 29, wherein the administration of said pharmacological agent is followed by gas/fluid exchange in the vitreous chamber.
79. A method for treating or reducing ocular neovascularization in a human subject, comprising:

    administering subretinally a pharmaceutical composition comprising a pharmaceutically effective amount of a recombinant virus comprising a nucleic acid encoding a VEGF inhibitor to a human subject in need of a treatment.

80. A method for treating or reducing ocular neovascularization in a human subject, 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.

81. A method for preventing ocular neovascularization in a human subject, 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.

82. A method for treating or reducing choroidal neovascularization in a human subject 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.

83. A method for preventing choroidal neovascularization in a human subject with dry 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.

84. The method of claim 79, 80, 81, 82 or 83, wherein said virus is selected from 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.

85. The method of claim 79, 80, 81, 82 or 83, wherein said AAV is selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, and hybrids thereof.

86. The method of claim 79, 80, 81, 82 or 83wherein the recombinant virus is generated from a plasmid comprising a kanamycin resistance marker.

87. The method of claim 79, 80, 81, 82 or 83wherein the recombinant virus is generated from a plasmid comprising an ampicillin resistance marker.

88. The method of claim 79, 80, 81, 82 or 83 wherein the recombinant virus is generated from a plasmid comprising a T7 RNA polymerase promoter sequence.

89. The method of claim 79, 80, 81, 82 or 83 wherein the recombinant virus is generated from a plasmid not comprising a T7 RNA polymerase promoter sequence.
90. The method of claim 79, 80, 81, 82 or 83, wherein said recombinant virus comprises an ampicillin resistance marker.
91. The method of claim 79, 80, 81, 82 or 83 wherein said recombinant virus comprises a T7 RNA polymerase promoter sequence.
92. The method of claim 79, 80, 81, 82 or 83 wherein said recombinant virus does not comprise a T7 RNA polymerase promoter sequence.
93. The method of claim 79, 80, 81, 82 or 83, wherein said 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.
94. The method of claim 79, 80, 81, 82 or 83, wherein said recombinant virus comprises an enhancer.
95. The method of claim 79, 80, 81, 82 or 83, wherein said recombinant virus comprises a chimeric intron.
96. The method of claim 79, 80, 81, 82 or 83, wherein said recombinant virus comprises a SV40 poly A sequence.
97. The method of claim 79, 80, 81, 82 or 83, wherein said recombinant virus comprises a human sFLT-1 protein or a functional fragment thereof.
98. The method of claim 79, 80, 81, 82 or 83, wherein said recombinant virus comprises a regulatory nucleic acid fragment that is capable of directing selective expression of the sFLT-1 protein in an eye cell.
99. The method of claim 79, 80, 81, 82 or 83, wherein said pharmaceutical composition comprises about 1×10^{6} to about 1×10^{15} recombinant viruses.
100. The method of claim 79, 80, 81, 82 or 83, wherein said pharmaceutical composition comprises about 1×10^{7} to about 1×10^{14} recombinant viruses.
101. The method of claim 79, 80, 81, 82 or 83, wherein said pharmaceutical composition comprises about 1×10^{8} to about 1×10^{13} recombinant viruses.
102. The method of claim 79, 80, 81, 82 or 83, wherein said pharmaceutical composition comprises about 1×10^{9} to about 1×10^{12} recombinant viruses.

103. The method of claim 79, 80, 81, 82 or 83, wherein said pharmaceutical composition comprises about 1×10^{10} to about 1×10^{12} recombinant viruses.

104. The method of claim 79, 80, 81, 82 or 83, wherein said pharmaceutical composition is administered via subretinal injection.

105. The method of claim 79, 80, 81, 82 or 83, further comprising administering to the human subject a pharmaceutically effective amount of a VEGF inhibitor.

106. The method of claim 105, wherein said VEGF inhibitor comprises an antibody against VEGF or a functional fragment thereof.

107. The method of claim 105, wherein said VEGF inhibitor comprises ranibizumab.

108. The method of claim 105, wherein said pharmaceutical composition is administered within 90 days of the administering of said VEGF inhibitor.

109. The method of claim 105, wherein said pharmaceutical composition is administered at least 5, 6, 7, or 8 days after the administering of said VEGF inhibitor.

110. The method of claim 105, wherein said VEGF inhibitor is administered at least 1 time prior to administering the said pharmaceutical composition.

111. The method of claim 105, wherein said VEGF inhibitor is administered for at least 1 time following administering of said pharmaceutical composition.

112. The method of claim 105, wherein said VEGF inhibitor is administered at least 2 times within 90 days of the administering of said VEGF inhibitor.

113. The method of claim 105, wherein said VEGF inhibitor is administered over a period of 6 to 7 weeks.

114. The method of claim 82, wherein said AMD is wet AMD.

115. The method of claim 81 wherein said human subject is at risk for developing age-related macular degeneration.

116. The method of claim 81 wherein said human subject presents symptoms of early stage age-related macular degeneration.

117. The method of claim 80 or 82 wherein said human subject has been previously treated with a different VEGF inhibitor for the treatment of AMD.

118. The method of claims 81 or 83 wherein the treated eye of said human subject has not been previously treated with a different VEGF inhibitor for the treatment of AMD.

119. The method of claim 80 or 82 wherein at least 5 treatments of a VEGF inhibitor for the treatment of AMD have been previously administered to said human subject.

120. The method of claim 80 or 82 wherein at least 10 treatments of a VEGF inhibitor for the treatment of AMD have been previously administered to said human subject.

121. The method of claim 80 or 82 wherein at least 15 treatments of a VEGF inhibitor for the treatment of AMD have been previously administered to said human subject.

122. The method of claim 80 or 82 wherein at least 20 treatments of a VEGF inhibitor for the treatment of AMD have been previously administered to said human subject.

123. The method of claim 79, 80, 81, 82 or 83 wherein best corrected visual acuity (BCVA), as measured by ETDRS (Early Treatment Diabetic Retinopathy Study) letters, decreased by fewer than 15 letters after said treatment with said pharmaceutical composition.

124. The method of claim 79, 80, 81, 82 or 83 wherein best corrected visual acuity (BCVA), as measured by ETDRS (Early Treatment Diabetic Retinopathy Study) letters, improved by less than 2 lines after said treatment with ranibizumab.

125. The method of claim 79, 80, 81, 82 or 83 wherein said human subject is at risk for developing dry age-related macular degeneration

126. The method of claim 79, 80, 81, 82 or 83 wherein said human subject presents symptoms of early stage dry age-related macular degeneration.

127. The method of claim 79, 80, 81, 82 or 83, wherein said treatment is administered at a frequency of less than once per 12 months.

128. The method of claim 79, 80, 81, 82 or 83, wherein said treatment is administered at a frequency of less than once per 6 months.

129. The method of claim 79, 80, 81, 82 or 83, wherein said treatment is administered at a frequency of less than once per 18 months.

130. The method of claim 79, 80, 81, 82 or 83 wherein said administering step is carried out in said human subject where subject is age 20 years or older.

131. The method of claim 79, 80, 81, 82 or 83 wherein said administering step is carried out in said human subject where subject is age is 40 years or older.

132. The method of claim 79, 80, 81, 82 or 83 wherein said administering step is carried out in said human subject where subject is age is 50 years or older.

133. The method of claim 79, 80, 81, 82 or 83 wherein said administering step is carried out in said human subject where subject is age is 65 years or older.

134. The method of claim 79, 80, 81, 82 or 83, wherein said administration is to a site that does not include the fovea.

135. The method of claim 79, 80, 81, 82 or 83, wherein said administration is in the central retina adjacent to the fovea.

136. The method of claim 80, wherein administration is to one or more cells of the subretinal space of the central retina.

137. The method of claim 79, 80, 81, 82 or 83, wherein administration is to one or more cells of the subretinal space of the outer macula.

138. The method of claim 79, 80, 81, 82 or 83, wherein said administration is to one or more cells of the subretinal space of the inner macula.

139. The method of claim 79, 80, 81, 82 or 83, wherein said administration is to retinal pigment epithelial cells.

140. The method of claim 79, 80, 81, 82 or 83, wherein said administration does not adversely affect central retinal function or central retinal structure.

141. The method of claim 79, 80, 81, 82 or 83, wherein said administering step is carried out simultaneously in both eyes.

142. The method of claim 79, 80, 81, 82 or 83, wherein said administering step is carried out sequentially in both eyes.

143. The method of claim 79, 80, 81, 82 or 83 wherein said administering step is carried out in one eye.

144. The method of claim 79, 80, 81, 82 or 83 wherein administering step is carried out in one eye when fellow eye presents symptoms of AMD.

145. The method of claim 79, 80, 81, 82 or 83 wherein said administering step is carried out in said human subject resistant to penicillin.

146. The method of claim 79, 80, 81, 82 or 83 wherein said administering step is carried out in said human subject sensitive to penicillin.

147. The method of claim 79, 80, 81, 82 or 83 wherein said administering step is carried out in said human subject allergic to penicillin.

148. The method of claim 79, 80, 81, 82 or 83 wherein said administering step is carried out in said human subject not allergic to penicillin.

149. The method of claim 79, 80, 81, 82 or 83, wherein no vector is detected in said human subject's blood, saliva or urine samples, 3, 7, 14, 21 or 30 days after administering said pharmaceutical composition.

150. The method of claim 149, wherein the presence of said viral vector is detected by qPCR or ELISA.

151. The method of claim 79, 80, 81, 82 or 83, wherein said sFLT-1 protein levels in the vitreous of said human subject is about 500 – 5,000 pg/ml, 7, 14, 21 or 30, 60, 90, 120, 150, 180, 270, 365 days after administering said pharmaceutical composition.

152. The method of claim 79, 80, 81, 82 or 83, wherein said sFLT-1 protein levels in the vitreous of said human subject is about 600 – 4,000 pg/ml, 7, 14, 21 or 30, 60, 90, 120, 150, 180, 270, 365 days after administering said pharmaceutical composition.

153. The method of claim 79, 80, 81, 82 or 83, wherein said sFLT-1 protein levels in the vitreous of said human subject is about 800 – 3,000 pg/ml, 7, 14, 21 or 30, 60, 90, 120, 150, 180, 270, 365 days after administering said pharmaceutical composition.

154. The method of claim 79, 80, 81, 82 or 83, wherein said sFLT-1 protein levels in the vitreous of said human subject is about 900 – 2,000 pg/ml, 7, 14, 21 or 30, 60, 90, 120, 150, 180, 270, 365 days after administering said pharmaceutical composition.

155. The method of claim 79, 80, 81, 82 or 83, wherein said sFLT-1 protein levels in the vitreous of said human subject is about 1,000 – 1,800 pg/ml, 7, 14, 21 or 30, 60, 90, 120, 150, 180, 270, 365 days after administering said pharmaceutical composition.

156. The method of claim 79, 80, 81, 82 or 83, wherein said sFLT-1 protein levels in the vitreous of said human subject is about 4,00 – 1,000 pg/ml, 7, 14, 21 or 30, 60, 90, 120, 150, 180, 270, 365 days after administering said pharmaceutical composition.

157. The method of claim 79, 80, 81, 82 or 83, wherein said human subject shows no clinically significant retinal toxicity as assessed by serial ophthalmic examinations over least a two months period.

158. The method of claim 79, 80, 81, 82 or 83, wherein no superficial, anterior segment or vitreous inflammatory signs are present in said human subject over least a two months period.

159. The method of claim 79, 80, 81, 82 or 83, wherein said human subject does not require rescue treatment at least 120 days post said administering.

160. The method of claim 79, 80, 81, 82 or 83, wherein 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 120 days post said administering.

161. The method of claim 79, 80, 81, 82 or 83 wherein no inflammation of the vitreous is observed by biomicroscopy (BE) and indirect opthalmoscopy (IOE) following the administering step.

162. The method of claim 79, 80, 81, 82 or 83 wherein no cytotoxic T cell response follows the administering step.

163. The method of claim 79, 80, 81, 82 or 83 wherein no cytotoxic T cell response within 10% of normal range follows the administering step.

164. The method of claim 79, 80, 81, 82 or 83 wherein best corrected visual acuity (BCVA), as measured by ETDRS (Early Treatment Diabetic Retinopathy Study) letters, improves following the administering step.

165. The method of claim 79, 80, 81, 82 or 83 wherein BCVA is improved by 1 line or more.

166. The method of claim 79, 80, 81, 82 or 83 wherein BCVA is improved by at least 2 lines or more.

167. The method of claim 79, 80, 81, 82 or 83 wherein BCVA is improved by at least 3 lines or more.

168. The method of claim 79, 80, 81, 82 or 83 wherein BCVA is improved by at least 4 lines or more.

169. The method of claim 79, 80, 81, 82 or 83 wherein BCVA is improved by at least 5 lines or more.

170. The method of claim 79, 80, 81, 82 or 83 wherein a reduction in neovascularization as observed by a Fluorscein Angiography (FA) follows the administering step.

171. The method of claim 79, 80, 81, 82 or 83 wherein frequency of administration of other anti-VEGF therapy is reduced.

172. The method of claim 79, 80, 81, 82 or 83 wherein frequency of administration other anti-VEGF therapy is less than monthly.

173. The method of claim 79, 80, 81, 82 or 83 wherein frequency of administration other anti-VEGF therapy is less than 3 months.

174. The method of claim 79, 80, 81, 82 or 83 wherein frequency of administration other anti-VEGF therapy is less than 6 months.

175. The method of claim 79, 80, 81, 82 or 83 wherein frequency of administration other anti-VEGF therapy is less than 1 year.

176. A unit dose of pharmaceutical composition comprising about 1×10^6 to 1×10^{15} recombinant viruses, wherein each said recombinant virus comprises a nucleic acid encoding soluble Fms-related tyrosine kinase-1 (sFLT-1) protein.

177. A unit dose of pharmaceutical composition of claim 176, comprising about 1×10^{10} to about 3×10^{12} recombinant viruses.

178. A unit dose of pharmaceutical composition of claim 176, comprising about 1×10^7 to about 1×10^{14} recombinant viruses.

179. A unit dose of pharmaceutical composition of claim 176, comprising about 1×10^8 to about 1×10^{13} recombinant viruses.

180. A unit dose of pharmaceutical composition of claim 176, comprising about 1×10^9 to about 1×10^{12} recombinant viruses.

181. A unit dose of pharmaceutical composition of claim 176, comprising about 1×10^{10} to about 1×10^{12} recombinant viruses.

182. The unit dose of pharmaceutical composition of claim 176, wherein said sFLT-1 protein comprises human sFLT-1 protein or a functional fragment thereof.

183. The unit dose of pharmaceutical composition of claim 176, wherein said virus is selected from 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.

184. The unit dose of pharmaceutical composition of claim 176, wherein said AAV is selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, and hybrids thereof.

185. The unit dose of pharmaceutical composition of claim 176, wherein said 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.

186. The unit dose of pharmaceutical composition of claim 176, wherein said recombinant virus comprises an enhancer.

187. The unit dose of pharmaceutical composition of claim 176, wherein said recombinant virus comprises a intron.

188. The unit dose of pharmaceutical composition of claim 176, wherein said recombinant virus comprises a SV40 poly A.

189. The unit dose of pharmaceutical composition of claim 176, wherein said recombinant virus comprises a regulatory nucleic acid fragment that is capable of directing selective expression of the sFLT-1 protein in an eye cell.

190. The unit dose of pharmaceutical composition of claim 176, wherein said recombinant virus is formulated with a surfactant.

191. The unit dose of pharmaceutical composition of claim 168, wherein said recombinant virus is manufactured using producer-clone methods.

192. The unit dose of pharmaceutical composition of claim 168, wherein said recombinant virus is manufactured using recombinant baculovirus.

193. The unit dose of pharmaceutical composition of claim 176, wherein said recombinant virus is manufactured using Ad-AAV.

194. The unit dose of pharmaceutical composition of claim 176, wherein said recombinant virus is manufactured using HEK 293 cells.

195. The unit dose of pharmaceutical composition of claim 176, wherein said recombinant virus is manufactured using insect cells.

196. The unit dose of pharmaceutical composition of claim 176, wherein said recombinant virus is manufactured using herpes-helper virus.

197. A method of treating, reducing, or preventing ocular neovascularization or macular edema in a human subject, comprising administering subretinally a pharmaceutical composition comprising a pharmaceutically effective amount of recombinant AAV gene delivery vector which directs the expression of an anti-angiogenic factor, such that administration of said vector inhibits neovascularization or macular edema in a human subject.

198. The method of claim 79, 80, 81, 82 or 83 wherein said administering step is carried out in said human subject with no history of infection with hepatitis B or hepatitis C.

199. The method of claim 79, 80, 81, 82 or 83, wherein said sFLT-1 protein levels in the vitreous of said human subject is elevated when measured at 7, 14, 21 or 30, 60, 90, 120, 150, 180, 270 or 365 days after administering said pharmaceutical composition.

SEQUENCE LISTING

<110> CONSTABLE, IAN J.
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<120> TREATMENT OF AMD USING AAV SFLT-1

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ggtttcgcca cctctgactt gagcgtcgat ttttgtgatg ctcgtcaggg gggcggagcc 180

tatggaaaaa cgccagcaac gcggcctttt tacggttcct ggccttttgc tggccttttg 240

ctcacatgtt ctttcctgcg ttatcccctg attctgtgga taaccgtatt accgcctttg 300

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gtaactggct tcagcagagc gcagatacca aatactgttc ttctagtgta gccgtagtta 180

ggccaccact tcaagaactc tgtagcaccg cctacatacc tcgctctgct aatcctgtta 240

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cgcacgaggg agcttccagg gggaaacgcc tggtatcttt atagtcctgt cgggtttcgc 540

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actcttttttc cgaaggtaac tggcttcagc agagcgcaga taccaaatac tgtccttcta 180

gtgtagccgt agttaggcca ccacttcaag aactctgtag caccgcctac atacctcgct 240

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<400> 15
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agcggtggtt tgtttgccgg atcaagagct accaactctt tttccgaagg taactggctt 120

cagcagagcg cagataccaa atactgtcct tctagtgtag ccgtagttag gccaccactt 180

caagaactct gtagcaccgc ctacatacct cgctctgcta atcctgttac cagtggctgc 240

tgccagtggc gataagtcgt gtcttaccgg gttggactca agacgatagt taccggataa 300

ggcgcagcgg tcgggctgaa cgggggttc gtgcacacag cccagcttgg agcgaacgac 360

ctacaccgaa ctgagatacc tacagcgtga gctatgagaa agcgccacgc ttcccgaagg 420

gagaaaggcg gacaggtatc cggtaagcgg cagggtcgga acaggagagc gcacgaggga 480

gcttccaggg ggaaacgcct ggtatcttta tagtcctgtc gggtttcgcc acctctgact 540

tgagcgtcga tttttgtgat gctcgtcagg ggggcggagc ctatggaaa 589

<210> 16
<211> 459
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 16
tttacgcgcc ctgtagcggc gcattaagcg cggcgggtgt ggtggttacg cgcagcgtga 60

ccgctacact tgccagcgcc ctagcgcccg ctcctttcgc tttcttccct tcctttctcg 120

ccacgttcgc cggctttccc cgtcaagctc taaatcgggg gctcccttta gggttccgat 180

ttagtgcttt acggcacctc gaccccaaaa aacttgattt gggtgatggt tcacgtagtg 240

ggccatcgcc ctgatagacg gtttttcgcc ctttgacgtt ggagtccacg ttctttaata 300

gtggactctt gttccaaact tgaacaacac tcaaccctat ctcgggctat tcttttgatt 360

tataagggat tttgccgatt tcggcctatt ggttaaaaaa tgagctgatt taacaaaaat 420

ttaacgcgaa ttttaacaaa atattaacgt ttacaattt 459

<210> 17
<211> 602
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 17
atgcattagt tattaatagt aatcaattac ggggtcatta gttcatagcc catatatgga 60

gttccgcgtt acataactta cggtaaatgg cccgcctggc tgaccgccca acgacccccg 120

cccattgacg tcaataatga cgtatgttcc catagtaacg ccaataggga ctttccattg 180

acgtcaatgg gtggagtatt tacggtaaac tgcccacttg gcagtacatc aagtgtatca 240

tatgccaagt acgccccta ttgacgtcaa tgacggtaaa tggcccgcct ggcattatgc 300

ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat tagtcatcgc 360

tattaccatg gtgatgcggt tttggcagta catcaatggg cgtggatagc ggtttgactc 420

acggggattt ccaagtctcc accccattga cgtcaatggg agtttgtttt ggcaccaaaa 480

tcaacgggac tttccaaaat gtcgtaacaa ctccgcccca ttgacgcaaa tgggcggtag 540

gcgtgtacgg tgggaggtct atataagcag agctggttta gtgaaccgtc agatccgcta 600

gc 602

<210> 18
<211> 741
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 18
tcaatattgg ccattagcca tattattcat tggttatata gcataaatca atattggcta 60

ttggccattg catacgttgt atctatatca taatatgtac atttatattg gctcatgtcc 120

aatatgaccg ccatgttggc attgattatt gactagttat taatagtaat caattacggg 180

gtcattagtt catagcccat atatggagtt ccgcgttaca taacttacgg taaatggccc 240

gcctggctga ccgcccaacg acccccgccc attgacgtca ataatgacgt atgttcccat 300

agtaacgcca atagggactt tccattgacg tcaatgggtg gagtatttac ggtaaactgc 360

ccacttggca gtacatcaag tgtatcatat gccaagtccg ccccctattg acgtcaatga 420

cggtaaatgg cccgcctggc attatgccca gtacatgacc ttacgggact ttcctacttg 480

gcagtacatc tacgtattag tcatcgctat taccatggtg atgcggtttt ggcagtacac 540

caatgggcgt ggatagcggt ttgactcacg gggatttcca agtctccacc ccattgacgt 600

caatgggagt ttgttttggc accaaaatca acgggacttt ccaaaatgtc gtaataaccc 660

cgccccgttg acgcaaatgg gcggtaggcg tgtacggtgg gaggtctata taagcagagc 720

tcgtttagtg aaccgtcaga t 741

<210> 19
<211> 664
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 19
acgcgtacta gttattaata gtaatcaatt acggggtcat tagttcatag cccatatatg 60

gagttccgcg ttacataact tacggtaaat ggcccgcctg gctgaccgcc caacgacccc 120

cgcccattga cgtcaataat gacgtatgtt cccatagtaa cgtcaatagg gactttccat 180

tgacgtcaat gggtggagta tttacggtaa actgcccact tggcagtaca tcaagtgtat 240

catatgccaa gtacgccccc tattgacgtc aatgacggta aatggcccgc ctggcattat 300

gcccagtaca tgaccttatg ggactttcct acttggcagt acatctacgt attagtcatc 360

gctattacca tggtgatgcg gttttggcag tacatcaatg ggcgtggata gcggtttgac 420

tcacggggat ttccaagtct ccaccccatt gacgtcaatg ggagtttgtt ttgcaccaaa 480

atcaacggga ctttccaaaa tgtcgtaaca actccgcccc attgacgcaa atgggcggta 540

ggcgtgtacg gtgggaggtc tatataagca gagctcgttt agtgaaccgt cagatcgcct 600

ggagacgcca tccacgctgt tttgacctcc atagaagaca ccgggaccga tccagcctcc 660

gtac 664

<210> 20
<211> 663
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 20
acgcgtggag ctagttatta atagtaatca attacggggt cattagttca tagcccatat 60

atggagttcc gcgttacata acttacggta aatggcccgc ctggctgacc gcccaacgac 120

ccccgcccat tgacgtcaat aatgacgtat gttcccatag taacgtcaat agggactttc 180

cattgacgtc aatgggtgga gtatttacgg taaactgccc acttggcagt acatcaagtg 240

tatcatatgc caagtacgcc ccctattgac gtcaatgacg gtaaatggcc cgcctggcat 300

tatgcccagt acatgacctt atgggacttt cctacttggc agtacatcta cgtattagtc 360

atcgctatta ccatggtgat gcggttttgg cagtacatca atgggcgtgg atagcggttt 420

gactcacggg gatttccaag tctccacccc attgacgtca atgggagttt gttttgcacc 480

aaaatcaacg ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg 540

gtaggcgtgt acggtgggag gtctatataa gcagagctcg tttagtgaac cgtcagatcg 600

cctggagacg ccatccacgc tgttttgacc tccatagaag acaccgggac cgatccagcc 660

tcc 663

<210> 21
<211> 589
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 21
tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata tggagttccg 60

cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt 120

gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca 180

atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc 240

aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta 300

catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tcgctattac 360

catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg actcacgggg 420

atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc aaaatcaacg 480

ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggcg gtaggcgtgt 540

acggtgggag gtctatataa gcagagctgg tttagtgaac cgtcagatc 589

<210> 22
<211> 670
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 22
gcggccgcac gcgtggagct agttattaat agtaatcaat tacggggtca ttagttcata 60

gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgcct ggctgaccgc 120

ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta acgtcaatag 180

ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac ttggcagtac 240

atcaagtgta tcatatgcca agtacgcccc ctattgacgt caatgacggt aaatggcccg 300

cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag tacatctacg 360

tattagtcat cgctattacc atggtgatgc ggttttggca gtacatcaat gggcgtggat 420

agcggtttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat gggagtttgt 480

tttgcaccaa aatcaacggg actttccaaa atgtcgtaac aactccgccc cattgacgca 540

aatgggcggt aggcgtgtac ggtgggaggt ctatataagc agagctcgtt tagtgaaccg 600

tcagatcgcc tggagacgcc atccacgctg ttttgacctc catagaagac accgggaccg 660

atccagcctc 670

<210> 23
<211> 490
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 23
ggcgaccgcc cagcgacccc cgcccgttga cgtcaatagt gacgtatgtt cccatagtaa 60

cgccaatagg gactttccat tgacgtcaat gggtggagta tttacggtaa actgcccact 120

tggcagtaca tcaagtgtat catatgccaa gtccgccccc tattgacgtc aatgacggta 180

aatggcccgc ctagcattat gcccagtaca tgaccttacg ggagtttcct acttggcagt 240

acatctacgt attagtcatc gctattacca tggtgatgcg gttttggcag tacaccaatg 300

ggcgtggata gcggtttgac tcacggggat ttccaagtct ccaccccatt gacgtcaatg 360

ggagtttgtt ttggcaccaa aatcaacggg actttccaaa atgtcgtaat aaccccgccc 420

cgttgaccca aatgggcggt aggcgtgtac ggtgggaggt ctatatagca gagctcgttt 480

agtgaaccgt 490

<210> 24
<211> 887
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 24
ttagtcatat gttacttggc agaggccgca tggaaagtcc ctggacgtgg gacatctgat 60

taatacgtga ggaggtcagc catgttcttt ttggcaaagg actacggtca ttggacgttt 120

gattggcatg ggatagggtc agccagagtt aacagtgttc ttttggcaaa gggatacgtg 180

gaaagccccg ggccatttac agtaaactga tacggggaca aagcacagcc atatttagtc 240

atgtactgct tggcagaggg tctatggaaa gtccctggac gtgggacgtc tgattaatat 300

gaaagaaggt cagccagagg tagctgtgtc ctttttggca aagggatacg gttatgggac 360

gtttgattgg actgggatag ggtcagccag agttaacagt gttcttttgg caaaggaaac 420

gtggaaagtc ccgggccatt tacagtaaac tgatactggg acaaagtaca cccatattta 480

gtcatgttct ttttggcaaa gagcatctgg aaagtcccgg gcagcattat agtcacttgg 540

cagagggaaa gggtcactca gagttaagta catctttcca gggccaatat tccagtaaat 600

tacacttagt tttatgcaaa tcagccacaa aggggatttt cccggtcaat tatgactttt 660

tccttagtca tgcggtatcc aattactgcc aaattggcag tacatactag gtgattcact 720

gacatttggc cgtcctctgg aaagtccctg gaaaccgctc aagtactgta tcatggtgac 780

tttgcatttt tggagagcac gccccactcc accattggtc cacgtaccct atgggggagt 840

ggtttatgag tatataaggg gctccggttt agaagccggg cagagcg 887

<210> 25
<211> 935
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 25
attgacgtca ataatgacgt atgttcccat agtaacgcca atagggactt tccattgacg 60

tcaatgggtg gactatttac ggtaaactgc ccacttggca gtacatcaag tgtatcatat 120

gccaagtacg ccccctattg acgtcaatga cggtaaatgg cccgcctggc attatgccca 180

gtacatgacc ttatgggact ttcctacttg gcagtacatc tacgtattag tcatcgctat 240

taccatggtc gaggtgagcc ccacgttctg cttcactctc cccatctccc cccccctccc 300

acccccaatt ttgtatttat ttatttttta attattttgt gcagcgatgg gggcgggggg 360

gggggggggg cgcgcgccag gcggggcggg gcggggcgag gggcggggcg gggcgaggcg 420

gagaggtgcg gcggcagcca atcagagcgg cgcgctccga aagtttcctt ttatggcgag 480

gcggcggcgg cggcggccct ataaaaagcg aagcgcgcgg cgggcgggag tcgctgcgac 540

gctgccttcg ccccgtgccc cgctccgccg ccgcctcgcg ccgcccgccc cggctctgac 600

tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg ggctgtaatt 660

agcgcttggt ttaatgacgg cttgtttctt ttctgtggct gcgtgaaagc cttgaggggc 720

tccgggaggg ccctttgtgc gggggagcg gctcggggct gtccgcgggg ggacggctgc 780

cttcgggggg gacggggcag ggcggggttc ggcttctggc gtgtgaccgg cggctctaga 840

gcctctgcta accatgttca tgccttcttc tttttcctac agctcctggg caacgtgctg 900

gttattgtgc tgtctcatca ttttggcaaa gaatt 935

<210> 26
<211> 367
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 26
gtcgaggtga gccccacgtt ctgcttcact ctccccatct ccccccccctc cccaccccca 60

attttgtatt tatttatttt ttaattattt tgtgcagcga tgggggcggg gggggggggg 120

gcgcgcgcca ggcggggcgg ggcggggcga ggggcggggc ggggcgaggc ggagaggtgc 180

ggcggcagcc aatcagagcg gcgcgctccg aaagtttcct tttatggcga ggcggcggcg 240

gcggcggccc tataaaaagc gaagcgcgcg gcgggcggga gtcgctgcgt tgccttcgcc 300

ccgtgccccg ctccgcgccg cctcgcgccg cccgccccgg ctctgactga ccgcgttact 360

cccacag 367

<210> 27
<211> 278
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 27
tcgaggtgag ccccacgttc tgcttcactc tccccatctc ccccccctcc ccaccccccaa 60

ttttgtattt atttatttttt taattatttt gtgcagcgat gggggcgggg gggggggggg 120

ggcgcgcgcc aggcggggcg gggcggggcg aggggcgggg cggggcgagg cggagaggtg 180

cggcggcagc caatcagagc ggcgcgctcc gaaagtttcc ttttatggcg aggcggcggc 240

ggcggcggcc ctataaaaag cgaagcgcgc ggcgggcg 278

<210> 28
<211> 109
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 28
ccagaaaaag tcaacacact tgtcataaag tcccgacgaa gtaaaacaag cggaattaat 60

tcaatttggc caaaaaacct agtataaaga cgtgcatagt gtcgggaat 109

<210> 29
<211> 999
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 29
cttcctcacg ctgaacccct ttaaccgttt cagtggtcgt gagtcttcta atctgactgt 60

gtgacgatgt tttaaggatt tggaggattg aggaggatca cctggtcagg taaatctgaa 120

atatccggat tacatcggaa gttgagcaca cggaaaaaca aaagactctt attggattta 180

gatccgtcag ccacctgctg ctgctcttca tcatcaggcg tcttcatcgc cctgcagtgg 240

gcctgacaac agcttgtgtt tattacacta aaaactttat aaacccatca caaaccatat 300

cacacagcag ggacttacct cttcatctgt aagaaggatt tttagagttg gcagcagagc 360

aacagtcagc tctgttgcct cactaaaaga gatctttgtt tgaatctgtg acctgtccaa 420

gtgtacctcg cttctcaccc actgacctct ccacaacagt gagctggttg gcgggatgct 480

aatgtttcta gttattacgt gtaaccaaac ttaaagagta cagataaatc atttagcata 540

attaaagttt tactgtcatg ttattggctg ttaatatgat tgctgttgta agtatgtgtt 600

gatcactaac aatttaatta attaaatcaa tcattaaatt aagtttgttt ggaaaaagag 660

ggaaaactca tccactgacc acatggttct aggttcaatt ccttggagtt aaagggctaa 720

tcccagagcc atttaccaaa ataataaata aatatttaaa taagacgtgc atgcgactgc 780

ggtcaccttt aaagcacaaa gtttttttg agcagtgagg tgaactcggg tggatctgtg 840

tgttcacaga gaaaaccttc tgtaagcaga ttaaggagtc agaagttctt aatcctgaaa 900

gtttagaaaa atcccagcag cataatcttt gctgtaagtg gtttacgagc gtatataaga 960

ggctgacaca gcggcagcgg caaagagctc agggtcaca 999

<210> 30
<211> 626
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 30
aaatcaaaag tcctcaacct ggttggaaga atattggcac tgaatggtat caataaggtt 60

gctagagagg gttagaggtg cacaatgtgc ttccataaca ttttatactt ctccaatctt 120

agcactaatc aaacatggtt gaatactttg tttactataa ctcttacaga gttataagat 180

ctgtgaagac agggacaggg acaataccca tctctgtctg gttcataggt ggtatgtaat 240

agatattttt aaaaataagt gagttaatga atgagggtga gaatggaggc acagaggtat 300

taggggggagg tgggccccag agaatggtgc caaggtccag tggggtgact gggatcagct 360

caggcctgac gctggccact cccacctagc tcctttcttt ctaatctgtt ctcattctcc 420

ttgggaagga ttgaggtctc tggaaaacag ccaaacaact gttatgggaa cagcaagccc 480

aaataaagcc aagcatcagg gggatctgag agctgaaagc aacttctgtt ccccctccct 540

cagctgaagg ggtggggaag ggctcccaaa gccataactc cttttaaggg atttagaagg 600

cataaaaagg cccctggctg agaact 626

<210> 31
<211> 173
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 31
actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc 60

aaaatcaacg ggactttcca aaatgtcgta ataaccccgc cccgttgacg caaatgggcg 120

gtaggcgtgt acggtgggag gtctatataa gcagagctcg tttagtgaac cgt 173

<210> 32
<211> 113
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 32
aaaatcaacg ggactttcca aaatgtcgta ataaccccgc cccgttgacg caaatgggcg 60

gtaggcgtgt acggtgggag gtctatataa gcagagctcg tttagtgaac cgt 113

<210> 33
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 33
gtaatacgac tcactatagg g 21

<210> 34
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 34
taatacgact cactatagg 19

<210> 35
<211> 67
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 35
ccgattaatc ataaatatga aaaataattg ttgcatcacc cgccaatgcg tggcttaatg 60

cacatca 67

<210> 36
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 36
attaaccctc actaaaggg 19

<210> 37
<211> 87
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 37
ctagacaagg tcgaacgagg ggcatgaccc ggtgcggggc ttcttgcact cggcataggc 60

gagtgctaag aataacgttg gcactcg 87

<210> 38
<211> 1478
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 38
tattaggcga agaggcatct agtagtagtg gcagtggtga gaacgtgggc gctgctatag 60

tgaacaatct ccagtcgatg gttaagaaga agagtgacaa accagcagtg aatgacttgt 120

ctgggtccgt gaggaaaaga aagaagcccg acacaaagga cagtaacgtc aagaaaccca 180

agaaataggg gggacctgtt tagatgtata ggaataaaaa ctccgagatg atctcaatgt 240

gtaatggagt tgtaatattg caaaggggga aaatcaagac tcaaacgtgt gtatgagtga 300

gcgtacgtat atctccgaga gtagtatgac ataatgatga ctgtgaatca tcgtaatctc 360

acacaaaaac cccattgtcg gccatatacc acaccaagca acaccacata tcccccggaa 420

aaaaaaacgt gaaaaaaaga aacaatcaaa actacaacct actccttgat cacacagtca 480

ttgatcaagt tacagttcct gctagggaat gaccaaggta caaatcagca ccttaatggt 540

tagcacgctc tcttactctc tctcacagtc ttccggcccc tattcaaaat tctgcacttc 600

catttgaccc cagggttggg aaacagggcc acaaaagaaa aacccgacgt gaatgaaaaa 660

actaagaaaa gaaaaaaaat tatcacacca gaaatttacc taattgggta attcccatcg 720

gtgtttttcc tggattgtcg cacgcacgca tgctgaaaaa agtgttcgag ttttgctttt 780

gcctcggagt ttcacgcaag tttttcgatc tcggaaccgg agggcggtcg ccttgttgtt 840

tgtgatgtcg tgctttgggt gttctaatgt gctgttattg tgctcttttt ttttcttctt 900

ttttggtga tcatatgata ttgctcggta gattactttc gtgtgtaggt attcttttag 960

acgtttggtt attgggtaga tatgagagag agagagtggg tgggggagga gttggttgta 1020

ggagggaccc ctgggaggaa gtgtagttga gttttccctg acgaatgaaa atacgttttt 1080

gagaagataa tacaggaaag gtgtgtcggt gaatttccat ctatccgagg atatgagtgg 1140

aggagagtcg tgtgcgtgtg gttaatttag gatcagtgga acacacaaag taactaagac 1200

agagagacag agagaaaaat ctggggaaga gacaaagagt cagagtgtgt gagttattct 1260

gtattgtgaa atttttttgc ccaactacat aatattgctg aaactaattt tacttaaaaa 1320

gaaaagccaa caacgtcccc agtaaaactt ttctataaat atcagcagtt ttccctttcc 1380

tccattcctc ttcttgtctt ttttcttact ttccctttt tatacctttt cattatcatc 1440

ctttataatt gtctaaccaa caactatata tctatcaa 1478

<210> 39
<211> 412
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 39
cccacacacc atagcttcaa aatgtttcta ctccttttt actcttccag attttctcgg 60

actccgcgca tcgccgtacc acttcaaaac acccaagcac agcatactaa attttccctc 120

tttcttcctc tagggtgtcg ttaattaccc gtactaaagg tttggaaaag aaaaaagaga 180

ccgcctcgtt tctttttctt cgtcgaaaaa ggcaataaaa atttttatca cgtttctttt 240

tcttgaaatt ttttttttta gttttttttct ctttcagtga cctccattga tatttaagtt 300

aataaacggt cttcaatttc tcaagtttca gtttcatttt tcttgttcta ttacaacttt 360

ttttacttct tgttcattag aaagaaagca tagcaatcta atctaagggg cg 412

<210> 40
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 40
ttgacaatta atcatcggct cgtataat 28

<210> 41
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 41
ttcaaatatg tatccgctca tgagaca 27

<210> 42
<211> 143
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 42
aactacccgt aggtgtagtt ggcgcaagcg tccgattagc tcaggtttaa gatgtcgaga 60

gtgagagtgg gcggcttaac tttctcagtt aggcataaaa ttacgtctta aatctcgtag 120

cgactaattt aataaaaatt gga 143

<210> 43
<211> 419
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 43
gcgcagcacc atggcctgaa ataacctctg aaagaggaac ttggttaggt accttctgag 60

gcggaaagaa ccagctgtgg aatgtgtgtc agttagggtg tggaaagtcc ccaggctccc 120

cagcaggcag aagtatgcaa agcatgcatc tcaattagtc agcaaccagg tgtggaaagt 180

ccccaggctc cccagcaggc agaagtatgc aaagcatgca tctcaattag tcagcaacca 240

tagtcccgcc cctaactccg cccatcccgc ccctaactcc gcccagttcc gcccattctc 300

cgccccatgg ctgactaatt ttttttattt atgcagaggc cgaggccgcc tcggcctctg 360

agctattcca gaagtagtga ggaggctttt ttggaggcct aggcttttgc aaaaagctt 419

<210> 44
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 44
gacgcttttt atcgcaactc tctactgt 28

<210> 45
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 45
catgacaaaa acgcgtaaca aaagtgtct 29

<210> 46
<211> 1904
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 46
agccgaattc ctcctcattc ttctccaaac ctttattgag tacctactgt gtgctggaat 60

aagacaggca gggccatgcc ctcatgaagc tgacaatcct attggtgtga ccatccccag 120

gtgtgtccca ggtgtgttgc aggtgtgtcc gaggtatgcc ccagctgtcc caggtgtgcc 180

ccagctgtct cagatgtgcc ccagctgtcc caggtgtgtc acagctgcat tgcaggtgtg 240

ccccagttgc attccatgtg tgctccaagt gtgtaccagc tgtcccaggt gtgtctcagg 300

tgtgccccag ctgtatccca ggtgtgcctc agctgtctta ggtgtgtctc aggtgcatcc 360

caggtgtgtc tcagatgtgc cccagctgtc ccaggtgtgc cccagctgtc ccaggtgtgc 420

cccagctgtc tccagtgtgt cccagctgtg ccccaggtgt gtgtcctagg tgtgcctcag 480

ctgtctcagg tgtgccccag gcatatccca ggtgtgcccc agctgtccca ggtgtgtcct 540

acgtgtgcac cagctgtatc ccaggtgtgc cccaggtgtg tctcagatgg gtcccaagtg 600

ttccccaact gcatttcagg tgtctcaggt gtgcccaagc tgtcccaggt gtgtccaaga 660

tgtgccccag gtgtgtctca ggtgggtctc aagtgcccca gctgcatttc aggtgtctca 720

ggtgtgcccc ccagtgcatc ccaggtgtgt cccaggtgtg ccccaggtgc atcccaggtg 780

tgtcccaggt gtgccccagc tgtctcaggt gtctcaggtg tgccccaggc atatcccagg 840

tgtgcctcag ctctcccagg tgtgtcctac atgtgcacca gctgtatctc aggtgtgtct 900

caggtgtgcc ccagatgtgc ccccggtgtg tctcaggtgg gtcccaagtg ttccccagct 960

gcatttcaag tgtctcaggt gtgccccagg tgtgcccccg ctgtcccagg tgtgtccaag 1020

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ttccaggtgt gtcccaggtg agccccagct gtattccata tgcgtccctc tgagtggggc 1380

cttggtttga tgtagctccg gggatcttct gctccctggt cctggtgtca ccagcaactg 1440

cctcttgaca atcctgcctt gcctgcaaac cccaggtgag aagaagacaa atgactggga 1500

actgacccct cagtaagcgc tggtggtctc acctacagac ccccaggaag ctggtcactg 1560

tgggcttctt ttcctctcta aattcctatt atcaggtggt tttctttctc atttgctatt 1620

ttcttaaaaa taaaaatagg gaaaaacagc ctttgtaaat tacggtttct tccggctcca 1680

tcctctccgt caggcccaca tcccaaggaa acagcaggct tgagcctggc tgctgaagcc 1740

aggggctgga tggagcagct cagaacagag ctttgagtgc ctctccagcc aggggcccca 1800

gaagcctggt ggttgtttgt ccttctcagg ggaaaagtga ggcggcccct tggaggaagg 1860

ggccgggcag aatgatctaa tcggattcca agcagctcag ggga 1904

<210> 47
<211> 352
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 47
ctctgagctg cttccctact cacactctgt ccacaacccc attttcctga tcatgtagta 60

gaaagaaatg gaacacaatc tttgtaaata agcccttgta aacaagcaag agctacagtg 120

cttccacaag ccctactgca agccaggaat gggaacagtg gtgtgtgtgc agcaaatgcc 180

ctgagcaccc ctgtggattg gactcagaaa catggaagtg agggtaggag gggatgatct 240

aagtcctggg cccaattaag agatcagatg gtgaagggtt tgggggcctt taaggtaagg 300

aggcctgggc tgatcctgca ggctgatata aagtcctgta accccatagg ca 352

<210> 48
<211> 133
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 48
gtaagtatca aggttacaag acaggtttaa ggagaccaat agaaactggg cttgtcgaga 60

cagagaagac tcttgcgttt ctgataggca cctattggtc ttactgacat ccactttgcc 120

tttctctcca cag 133

<210> 49
<211> 221
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 49
agacatgata agatacattg atgagtttgg acaaaccaca actagaatgc agtgaaaaaa 60

atgctttatt tgtgaaattt gtgatgctat tgctttattt gtaaccatta taagctgcaa 120

taaacaagtt aacaacaaca attgcattca ttttatgttt caggttcagg gggagatgtg 180

ggaggttttt taaagcaagt aaaacctcta caaatgtggt a 221

<210> 50
<211> 222
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 50
cagacatgat aagatacatt gatgagtttg gacaaaccac aactagaatg cagtgaaaaa 60

aatgctttat ttgtgaaatt tgtgatgcta ttgctttatt tgtaaccatt ataagctgca 120

ataaacaagt taacaacaac aattgcattc attttatgtt tcaggttcag ggggagatgt 180

gggaggtttt ttaaagcaag taaaacctct acaaatgtgg ta 222

<210> 51
<211> 239
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 51
ggccgcttcg agcagacatg ataagataca ttgatgagtt tggacaaacc acaactagaa 60

tgcagtgaaa aaaatgcttt atttgtgaaa tttgtgatgc tattgcttta tttgtaacca 120

ttataagctg caataaacaa gttaacaaca acaattgcat tcattttatg tttcaggttc 180

agggggagat gtgggaggtt ttttaaagca agtaaaacct ctacaaatgt ggtaaaatc 239

<210> 52
<211> 222
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 52
taatcagcat accacatttg tagaggtttt acttgcttta aaaaacctcc cacacctccc 60

cctgaacctg aaacataaaa tgaatgcaat tgttgttgtt aacttgttta ttgcagctta 120

taatggttac aaataaagca atagcatcac aaatttcaca aataaagcat ttttttcact 180

gcattctagt tgtggtttgt ccaaactcat caatgtatct ta 222

<210> 53
<211> 142
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 53
aacttgttta ttgcagctta taatggttac aaataaagca atagcatcac aaatttcaca 60

aataaagcat ttttttcact gcattctagt tgtggtttgt ccaaactcat caatgtatct 120

tatcacgtct ggtcaggtgg ca 142

<210> 54
<211> 132
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 54
aacttgttta ttgcagctta taatggttac aaataaagca atagcatcac aaatttcaca 60

aataaagcat ttttttcact gcattctagt tgtggtttgt ccaaactcat caatgtatct 120

tatcacgtct gg 132

<210> 55
<211> 49
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 55
aataaaatat ctttattttc attacatctg tgtgttggtt ttttgtgtg 49

<210> 56
<211> 341
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 56
ctgcgcgctc gctcgctcac tgaggccgcc cgggcaaagc ccgggcgtcg ggcgaccttt 60

ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact 120

aggggttcct tgtagttaat gattaacccg ccatgctact tatctacgcg tagataagta 180

gcatggcggg ttaatcatta actacaagga acccctagtg atggagttgg ccactccctc 240

tctgcgcgct cgctcgctca ctgaggccgg gcgaccaaag gtcgcccgac gcccgggctt 300

tgcccgggcg gcctcagtga gcgagcgagc gcgcagcctt a 341

<210> 57
<211> 344
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

polynucleotide

<400> 57
aggctgcgcg ctcgctcgct cactgaggcc gcccgggcaa agcccgggcg tcgggcgacc 60

tttggtcgcc cggcctcagt gagcgagcga gcgcgcagag agggagtggc caactccatc 120

actaggggtt ccttgtagtt aatgattaac ccgccatgct acttatctac gcgtagataa 180

gtagcatggc gggttaatca ttaactacaa ggaaccccta gtgatggagt tggccactcc 240

ctctctgcgc gctcgctcgc tcactgaggc cgggcgacca aaggtcgccc gacgcccggg 300

ctttgcccgg gcggcctcag tgagcgagcg agcgcgcagc ctta 344

<210> 58
<211> 128
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 58
aggaacccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctcactgagg 60

ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg ggcggcctca gtgagcgagc 120

gagcgcgc 128

<210> 59
<211> 130
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 59
aggaacccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctcactgagg 60

ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg ggcggcctca gtgagcgagc 120

gagcgcgcag 130

<210> 60
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 60
ttatgaagat ccctcgacct gcagcccaag cttggcgtaa tcatg 45

<210> 61
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 61
gataaggatc ttcctagagc atggcta 27

<210> 62
<211> 273
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 62
atgtctgccc gtatttcgcg taaggaaatc cattatgtac tatttaaaaa acacaaactt 60

ttggatgttc ggtttattct ttttctttta ctttttttatc atgggagcct acttcccgtt 120

tttcccgatt tggctacatg acatcaacca tatcagcaaa agtgatacgg gtattatttt 180

tgccgctatt tctctgttct cgctattatt ccaaccgctg tttggtctgc tttctgacaa 240

actcggcctc gactctaggc ggccgcgggg atc 273

<210> 63
<211> 141
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 63
ggaggggtgg agtcgtgacg tgaattacgt catagggtta gggaggtcct gtattagagg 60

tcacgtgagt gttttgcgac attttgcgac accatgtggt cacgctgggt atttaagccc 120

gagtgagcac gcagggtctc c 141

<210> 64
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 64
ggatccactc gagtggagct cgcgactagt cgattcgaat tcgatatcaa gcttatcgat 60

<210> 65
<211> 98
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 65
gcctgtacgg aagtgttact tctgctctaa aagctgcgga attgtacccg cggccgcaat 60

tcccggggat cgaaagagcc tgctaaagca aaaaagaa 98

<210> 66
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 66
ccgaacccga attctgcaga tatccagcac agtggcggcc gcttcgag 48

<210> 67
<211> 53
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 67
tagtttccat ggctacgtag ataagtagca tggcgggtta atcattaact aca 53

<210> 68
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 68
tgtagttaat gattaacccg ccatgctact tatctacgta gccatgctcg atctgaattc 60

ggta 64

<210> 69
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 69
ggccgcgggg atcc 14

<210> 70
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 70
ggttcgaaca g 11

<210> 71
<211> 120
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 71
catgttcatg ccttcttctt tttcctacag ctcctgggca acgtgctggt tattgtgctg 60

tctcatcatt ttggcaaaga attctgcagt cgacggtacc gcgggcccgg gatccaccgg 120

<210> 72
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 72
gcggccgcac gcgtgttact agttattaat 30

<210> 73
<211> 115
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 73
cactagaagc tttattgcgg tagtttatca cagttaaatt gctaacgcag tcagtgcttc 60

tgacacaaca gtctcgaact taagctgcag aagttggtcg tgaggcactg ggcag 115

<210> 74
<211> 68
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 74
gcctggagac gccatccacg ctgttttgac ctccatagaa gacaccggga ccgatccagc 60

ctccggac 68

<210> 75
<211> 133
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 75
gtgtccactc ccagttcaat tacagctctt aaggctagag tacttaatac gactcactat 60

aggctagcct cgagaattca cgcgtggtac cgagctcgga tccactagtc cagtgtggtg 120

gaattcgggc ggg 133

<210> 76
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 76
tgtatttaga aaaataaaca aataggggtt ccgcgcacat ttccccgaaa agtgccacct 60

ggtc 64

<210> 77
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 77
tagccatgct ctaggaagat cgtacc 26

<210> 78
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 78
gaattcgagc ttgcatgcct gcaggt 26

<210> 79
<211> 56
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 79
gtgtccactc ccagttcaat tacagctctt aaggctagag tacttctagc ctcgag 56

<210> 80
<211> 67

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 80
aaatcgataa ggatcctaga gcatggctac gtagataagt agcatggcgg gttaatcatt 60

aactaca 67

<210> 81
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 81
ggcccgggat cca 13

<210> 82
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 82
gttgaattcg atatcggatc catcgatacc gtcgacctcg agggggggcc cggtacc 57

<210> 83
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 83
caattcgccc tatagtgagt cgtattacgc gcgcagcggc cgac 44

<210> 84
<211> 56
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 84
tgtagttaat gattaacccg ccatgctact tatctacgta gccatgctct agatct 56

<210> 85
<211> 18
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 85
agcggccgca ctcctcag 18

<210> 86
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 86
atcgataccg tcgacccggg c 21

<210> 87
<211> 122
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 87
cagatcgcct ggagacgcca tccacgctgt tttgacctcc atagaagaca ccgggaccga 60

tccagcctcc ggactctaga ggatccggta ctcgaggaac tgaaaaacca gaaagttaac 120

tg 122

<210> 88
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 88
ccggaccacg tgcggaccga gcggccgc 28

<210> 89
<211> 115
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 89
gtgtccactc ccagttcaat tacagctctt aaggctagag tacttctagc ctcgagaatt 60

cacgcgtggt accgagctcg gatccactag tccagtgtgg tggaattcgg gcggg 115

<210> 90
<211> 67
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 90
ttatttgtga aatttgtgat gctattgctt tatttgtaac cattataagc tgcaataaac 60

aagttaa 67

<210> 91
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 91
taataataac cgggcaggcc 20

<210> 92
<211> 124
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 92
agccgcgaga cgggcgctca gggcgcgggg ccggcggcgg cgaacgagag gacggactct 60

ggcggccggg tcgttggccg cggggagcgc gggcaccggg cgagcaggcc gcgtcgcgct 120

cacc 124

<210> 93
<211> 194
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 93
ggggctcggg tgcagcggcc agcgggcctg gcggcgagga ttacccgggg aagtggttgt 60

ctcctggctg gagccgcgag acgggcgctc agggcgcggg gccggcggcg gcgaacgaga 120

ggacggactc tggcggccgg gtcgttggcc gggggagcgc gggcaccggg cgagcaggcc 180

gcgtcgcgct cacc 194

<210> 94
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 94
aggactcatt aaaaagtaac 20

<210> 95
<211> 197
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 95
ggggctcggg tgcagcggcc agcgggcgcc tggcggcgag gattacccgg ggaagtggtt 60

gtctcctggc tggagccgcg agacgggcgc tcagggcgcg gggccggcgg cggcgaacga 120

gaggacggac tctggcggcc gggtcgttgg ccgcggggag cgcgggcacc gggcgagcag 180

gccgcgtcgc gctcacc 197

<210> 96
<211> 1000
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 96
gggcgggtgc atcaatgcgg ccgaaaaaga cacggacacg ctcccctggg acctgagctg 60

gttcgcagtc ttcccaaagg tgccaagcaa gcgtcagttc ccctcaggcg ctccaggttc 120

agtgccttgt gccgagggtc tccggtgcct tcctagactt ctcgggacag tctgaagggg 180

tcaggagcgg cgggacagcg cgggaagagc aggcaagggg agacagccgg actgcgcctc 240

agtcctccgt gccaagaaca ccgtcgcgga ggcgcggcca gcttcccttg gatcggactt 300

tccgccccta gggccaggcg gcggagcttc agccttgtcc cttccccagt ttcgggcggc 360

ccccagagct gagtaagccg ggtggaggga gtctgcaagg atttcctgag cgcgatgggc 420

aggaggaggg gcaagggcaa gagggcgcgg agcaaagacc ctgaacctgc cggggccgcg 480

ctcccgggcc cgcgtcgcca gcacctcccc acgcgcgctc ggccccgggc cacccgccct 540

cgtcggcccc cgcccctctc cgtagccgca gggaagcgag cctgggagga agaagagggt 600

aggtggggag gcggatgagg ggtgggggac cccttgacgt caccagaagg aggtgccggg 660

gtaggaagtg ggctggggaa aggttataaa tcgcccccgc cctcggctgc tcttcatcga 720

ggtccgcggg aggctcggag cgcgccaggc ggacactcct ctcggctcct ccccggcagc 780

ggcggcggct cggagcgggc tccggggctc gggtgcagcg gccagcgggc gcctggcggc 840

gaggattacc cggggaagtg gttgtctcct ggctggagcc gcgagacggg cgctcagggc 900

gcggggccgg cggcggcgaa cgagaggacg gactctggcg gccgggtcgt tggccgcggg 960

gagcgcgggc accgggcgag caggccgcgt cgcgctcacc 1000

<210> 97
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 97
ttgcttgtta atcaataaac cgtttaattc gtttcagttg aactttggtc tctgcgtatt 60

tctttcttat c 71

<210> 98
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 98
attttgaagc gggaggtttg aacgcgcagc cgcc 34

<210> 99
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 99
ccggtcgcca cc 12

<210> 100
<211> 197
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 100
ggggctcggg tgcagcggcc agcgggcgcc tggcggcgag gattacccgg ggaagtggtt 60

gtctcctggc tggagccgcg agacgggcgc tcagggcgcg gggccggcgg cggcgaacga 120

gaggacggac tctggcggcc gggtctttgg ccgcggggag cgcgggcacc gggcgagcag 180

gccgcgtcgc gctcacc 197

<210> 101
<211> 382
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 101
ggggtggggg acccccttgac gtcaccagaa ggaggtgccg gggtaggaag tgggctgggg 60

aaaggttata aatcgccccc gccctcggct gctcttcatc gaggtccgcg ggaggctcgg 120

agcgcgccag gcggacactc ctctcggctc ctccccggca gcggcggcgg ctcggagcgg 180

gctccggggc tcgggtgcag cggccagcgg gcgcctggcg gcgaggatta cccggggaag 240

tggttgtctc ctggctggag ccgcgagacg ggcgctcagg gcgcggggcc ggcggcggcg 300

aacgagagga cggactctgg cggccgggtc gttggccgcg gggagcgcgg gcaccgggcg 360

agcaggccgc gtcgcgctca cc 382

<210> 102
<211> 2064
<212> DNA
<213> Homo sapiens

<400> 102
atggtcagct actgggacac cggggtcctg ctgtgcgcgc tgctcagctg tctgcttctc 60

acaggatcta gttcaggttc aaaattaaaa gatcctgaac tgagtttaaa aggcacccag 120

cacatcatgc aagcaggcca gacactgcat ctccaatgca ggggggaagc agcccataaa 180

tggtctttgc ctgaaatggt gagtaaggaa agcgaaaggc tgagcataac taaatctgcc 240

tgtggaagaa atggcaaaca attctgcagt actttaacct tgaacacagc tcaagcaaac 300

cacactggct tctacagctg caaatatcta gctgtaccta cttcaaagaa gaaggaaaca 360

gaatctgcaa tctatatatt tattagtgat acaggtagac ctttcgtaga gatgtacagt 420

gaaatccccg aaattataca catgactgaa ggaagggagc tcgtcattcc ctgccgggtt 480

acgtcaccta acatcactgt tactttaaaa aagtttccac ttgacacttt gatccctgat 540

ggaaaacgca taatctggga cagtagaaag ggcttcatca tatcaaatgc aacgtacaaa 600

gaaatagggc ttctgacctg tgaagcaaca gtcaatgggc atttgtataa gacaaactat 660

ctcacacatc gacaaaccaa tacaatcata gatgtccaaa taagcacacc acgcccagtc 720

aaattactta gaggccatac tcttgtcctc aattgtactg ctaccactcc cttgaacacg 780

agagttcaaa tgacctggag ttaccctgat gaaaaaaata agagagcttc cgtaaggcga 840

cgaattgacc aaagcaattc ccatgccaac atattctaca gtgttcttac tattgacaaa 900

atgcagaaca aagacaaagg actttatact tgtcgtgtaa ggagtggacc atcattcaaa 960

tctgttaaca cctcagtgca tatatatgat aaagcattca tcactgtgaa acatcgaaaa 1020

cagcaggtgc ttgaaaccgt agctggcaag cggtcttacc ggctctctat gaaagtgaag 1080

gcatttccct cgccggaagt tgtatggtta aaagatgggt tacctgcgac tgagaaatct 1140

gctcgctatt tgactcgtgg ctactcgtta attatcaagg acgtaactga agaggatgca 1200

gggaattata caatcttgct gagcataaaa cagtcaaatg tgtttaaaaa cctcactgcc 1260

actctaattg tcaatgtgaa accccagatt tacgaaaagg ccgtgtcatc gtttccagac 1320

ccggctctct acccactggg cagcagacaa atcctgactt gtaccgcata tggtatccct 1380

caacctacaa tcaagtggtt ctggcacccc tgtaaccata atcattccga agcaaggtgt 1440

gacttttgtt ccaataatga agagtccttt atcctggatg ctgacagcaa catgggaaac 1500

agaattgaga gcatcactca gcgcatggca ataatagaag gaaagaataa gatggctagc 1560

accttggttg tggctgactc tagaatttct ggaatctaca tttgcatagc ttccaataaa 1620

gttgggactg tgggaagaaa cataagcttt tatatcacag atgtgccaaa tgggtttcat 1680

gttaacttgg aaaaaatgcc gacggaagga gaggacctga aactgtcttg cacagttaac 1740

aagttcttat acagagacgt tacttggatt ttactgcgga cagttaataa cagaacaatg 1800

cactacagta ttagcaagca aaaaatggcc atcactaagg agcactccat cactcttaat 1860

cttaccatca tgaatgtttc cctgcaagat tcaggcacct atgcctgcag agccaggaat 1920

gtatacacag gggaagaaat cctccagaag aaagaaatta caatcagagg tgagcactgc 1980

aacaaaaagg ctgttttctc tcggatctcc aaatttaaaa gcacaaggaa tgattgtacc 2040

acacaaagta atgtaaaaca ttaa 2064

<210> 103
<211> 1453
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 103
aagcttgggc tgcaggtcga tcgactctag aggatcgatc cccgggcgag ctcgaattcg 60

caaccaccat ggtcagctac tgggacaccg gggtcctgct gtgcgcgctg ctcagctgtc 120

tgcttctcac aggatctagt tccggaggta gacctttcgt agagatgtac agtgaaatcc 180

ccgaaattat acacatgact gaaggaaggg agctcgtcat tccctgccgg gttacgtcac 240

ctaacatcac tgttacttta aaaaagtttc cacttgacac tttgatccct gatggaaaac 300

gcataatctg ggacagtaga aagggcttca tcatatcaaa tgcaacgtac aaagaaatag 360

ggcttctgac ctgtgaagca acagtcaatg ggcatttgta taagacaaac tatctcacac 420

atcgacaaac caatacaatc atagatgtgg ttctgagtcc gtctcatgga attgaactat 480

ctgttggaga aaagcttgtc ttaaattgta cagcaagaac tgaactaaat gtggggattg 540

acttcaactg ggaataccct tcttcgaagc atcagcataa gaaacttgta aaccgagacc 600

taaaaaccca gtctgggagt gagatgaaga aatttttgag caccttaact atagatggtg 660

taacccggag tgaccaagga ttgtacacct gtgcagcatc cagtgggctg atgaccaaga 720

agaacagcac atttgtcagg gtccatgaaa agggcccggg cgacaaaact cacacatgcc 780

caccgtgccc agcacctgaa ctcctggggg gaccgtcagt cttcctcttc cccccaaaac 840

ccaaggacac cctcatgatc tcccggaccc ctgaggtcac atgcgtggtg gtggacgtga 900

gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag gtgcataatg 960

ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc agcgtcctca 1020

ccgtcctgca ccaggactgg ctgaatggca aggagtacaa gtgcaaggtc tccaacaaag 1080

ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc cgagaaccac 1140

aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggtc agcctgacct 1200

gcctggtcaa aggcttctat cccagcgaca tcgccgtgga gtgggagagc aatgggcagc 1260

cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc ttcttcctct 1320

atagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc tcatgctccg 1380

tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg tctccgggta 1440

aatgagcggc cgc 1453

<210> 104
<211> 1377
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 104
atggtcagct actgggacac cggggtcctg ctgtgcgcgc tgctcagctg tctgcttctc 60

acaggatcta gttccggaag tgataccggt agacctttcg tagagatgta cagtgaaatc 120

cccgaaatta tacacatgac tgaaggaagg gagctcgtca ttccctgccg ggttacgtca 180

cctaacatca ctgttacttt aaaaaagttt ccacttgaca ctttgatccc tgatggaaaa 240

cgcataatct gggacagtag aaagggcttc atcatatcaa atgcaacgta caaagaaata 300

gggcttctga cctgtgaagc aacagtcaat gggcatttgt ataagacaaa ctatctcaca 360

catcgacaaa ccaatacaat catagatgtg gttctgagtc cgtctcatgg aattgaacta 420

tctgttggag aaaagcttgt cttaaattgt acagcaagaa ctgaactaaa tgtggggatt 480

gacttcaact gggaataccc ttcttcgaag catcagcata agaaacttgt aaaccgagac 540

ctaaaaaccc agtctgggag tgagatgaag aaatttttga gcaccttaac tatagatggt 600

gtaacccgga gtgaccaagg attgtacacc tgtgcagcat ccagtgggct gatgaccaag 660

aagaacagca catttgtcag ggtccatgaa aaggacaaaa ctcacacatg cccaccgtgc 720

ccagcacctg aactcctggg gggaccgtca gtcttcctct tcccccaaa acccaaggac 780

accctcatga tctcccggac ccctgaggtc acatgcgtgg tggtggacgt gagccacgaa 840

gaccctgagg tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca 900

aagccgcggg aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg 960

caccaggact ggctgaatgg caaggagtac aagtgcaagg tctccaacaa agccctccca 1020

gcccccatcg agaaaaccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac 1080

accctgcccc catcccggga tgagctgacc aagaaccagg tcagcctgac ctgcctggtc 1140

aaaggcttct atcccagcga catcgccgtg gagtgggaga gcaatgggca gccggagaac 1200

aactacaaga ccacgcctcc cgtgctggac tccgacggct ccttcttcct ctacagcaag 1260

ctcaccgtgg acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgcat 1320

gaggctctgc acaaccacta cacgcagaag agcctctccc tgtctccggg taaatga 1377

<210> 105
<211> 1444
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 105
aagcttgggc tgcaggtcga tcgactctag aggatcgatc cccgggcgag ctcgaattcg 60

caaccaccat ggtcagctac tgggacaccg gggtcctgct gtgcgcgctg ctcagctgtc 120

tgcttctcac aggatctagt tccggaggta gacctttcgt agagatgtac agtgaaatcc 180

ccgaaattat acacatgact gaaggaaggg agctcgtcat tccctgccgg gttacgtcac 240

ctaacatcac tgttacttta aaaaagtttc cacttgacac tttgatccct gatggaaaac 300

gcataatctg ggacagtaga aagggcttca tcatatcaaa tgcaacgtac aaagaaatag 360

ggcttctgac ctgtgaagca acagtcaatg ggcatttgta taagacaaac tatctcacac 420

atcgacaaac caatacaatc atagatatcc agctgttgcc caggaagtcg ctggagctgc 480

tggtagggga gaagctggtc ctcaactgca ccgtgtgggc tgagtttaac tcaggtgtca 540

cctttgactg ggactaccca gggaagcagg cagagcgggg taagtgggtg cccgagcgac 600

gctcccaaca gacccacaca gaactctcca gcatcctgac catccacaac gtcagccagc 660

acgacctggg ctcgtatgtg tgcaaggcca acaacggcat ccagcgattt cgggagagca 720

ccgaggtcat tgtgcatgaa aatggcccgg gcgacaaaac tcacacatgc ccaccgtgcc 780

cagcacctga actcctgggg ggaccgtcag tcttcctctt cccccccaaaa cccaaggaca 840

ccctcatgat ctcccggacc cctgaggtca catgcgtggt ggtggacgtg agccacgaag 900

accctgaggt caagttcaac tggtacgtgg acggcgtgga ggtgcataat gccaagacaa 960

agccgcggga ggagcagtac aacagcacgt accgtgtggt cagcgtcctc accgtcctgc 1020

accaggactg gctgaatggc aaggagtaca agtgcaaggt ctccaacaaa gccctcccag 1080

cccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca caggtgtaca 1140

ccctgccccc atcccgggat gagctgacca agaaccaggt cagcctgacc tgcctggtca 1200

aaggcttcta tcccagcgac atcgccgtgg agtgggagag caatgggcag ccggagaaca 1260

actacaagac cacgcctccc gtgctggact ccgacggctc cttcttcctc tatagcaagc 1320

tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgctcc gtgatgcatg 1380

aggctctgca caaccactac acgcagaaga gcctctccct gtctccgggt aaatgagcgg 1440

ccgc 1444

<210> 106
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 106

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Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr
            20                  25                  30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Val Leu Ile
        35                  40                  45

Tyr Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205
```

Phe Asn Arg Gly Glu Cys
210

<210> 107
<211> 642
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 107
gatattcagc tgacccagag cccgagcagc ctgagcgcga gcgtgggcga tcgcgtgacc 60

attacctgca gcgcgagcca ggatattagc aactatctga actggtatca gcagaaaccg 120

ggcaaagcgc cgaaagtgct gatttatttt accagcagcc tgcatagcgg cgtgccgagc 180

cgctttagcg gcagcggcag cggcaccgat tttaccctga ccattagcag cctgcagccg 240

gaagattttg cgacctatta ttgccagcag tatagcaccg tgccgtggac ctttggccag 300

ggcaccaaag tggaaattaa acgcaccgtg gcggcgccga gcgtgtttat ttttccgccg 360

agcgatgaac agctgaaaag cggcaccgcg agcgtggtgt gcctgctgaa caacttttat 420

ccgcgcgaag cgaaagtgca gtggaaagtg gataacgcgc tgcagagcgg caacagccag 480

gaaagcgtga ccgaacagga tagcaaagat agcacctata gcctgagcag caccctgacc 540

ctgagcaaag cggattatga aaaacataaa gtgtatgcgt gcgaagtgac ccatcagggc 600

ctgagcagcc cggtgaccaa aagctttaac cgcggcgaat gc 642

<210> 108
<211> 642
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 108
gatattcaat tgactcaatc tccttcttct ttgtctgctt ctgttggtga tcgtgttact 60

attacttgtt ctgcttctca agatatttct aattatttga attggtatca acaaaaacct 120

ggtaaagctc ctaaagtttt gatttatttt acttcttctt tgcattctgg tgttccttct 180

cgtttttctg gttctggttc tggtactgat tttactttga ctatttcttc tttgcaacct 240

gaagattttg ctacttatta ttgtcaacaa tattctactg ttccttggac ttttggtcaa 300

ggtactaaag ttgaaattaa acgtactgtt gctgctcctt ctgtttttat ttttcctcct 360

tctgatgaac aattgaaatc tggtactgct tctgttgttt gtttgttgaa taatttttat 420

cctcgtgaag ctaaagttca atggaaagtt gataatgctt tgcaatctgg taattctcaa 480

gaatctgtta ctgaacaaga ttctaaagat tctacttatt ctttgtcttc tactttgact 540

ttgtctaaag ctgattatga aaaacataaa gtttatgctt gtgaagttac tcatcaaggt 600

ttgtcttctc ctgttactaa atcttttaat cgtggtgaat gt 642

<210> 109
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 109

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Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
1               5                  10                  15

Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro
            20                  25                  30

Glu Leu Ser Leu Lys Gly Thr Gln His Ile Ala Gly Gln Thr Leu His
        35                  40                  45

Leu Gln Cys Arg Gly Glu Ala Ala Met Gln His Lys Trp Ser Leu Pro
    50                  55                  60

Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
65                  70                  75                  80

Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
                85                  90                  95

Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
            100                 105                 110

Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
        115                 120                 125

Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
    130                 135                 140

Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
145                 150                 155                 160

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
                165                 170                 175

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
            180                 185                 190

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
        195                 200                 205

Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
    210                 215                 220
```

Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
225 230 235 240

Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
245 250 255

Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
260 265 270

Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
275 280 285

Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
290 295 300

Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
305 310 315 320

Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val
325 330 335

Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
340 345 350

Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val
355 360 365

Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu
370 375 380

Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala
385 390 395 400

Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
405 410 415

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu
420 425 430

Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser
435 440 445

Arg Gln
450

<210> 110
<211> 375
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 110
atggccaagt tgaccagtgc cgttccggtg ctcaccgcgc gcgacgtcgc cggagcggtc 60

gagttctgga ccgaccggct cgggttctcc cgggacttcg tggaggacga cttcgccggt 120

gtggtccggg acgacgtgac cctgttcatc agcgcggtcc aggaccaggt ggtgccggac 180

aacaccctgg cctgggtgtg ggtgcgcggc ctggacgagc tgtacgccga gtggtcggag 240

gtcgtgtcca cgaacttccg ggacgcctcc gggccggcca tgaccgagat cggcgagcag 300

ccgtgggggc gggagttcgc cctgcgcgac ccggccggca actgcgtgca cttcgtggcc 360

gaggagcagg actga 375

<210> 111
<211> 375
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 111
atgtctaaat taacctctgc tgttccagtg ttaaccgccc gtgatgttgc cggtgcagtg 60

gaattttgga ctgaccgttt gggtttctca cgtgactttg tcgaagatga ttttgctggc 120

gttgtgcgtg atgacgtcac tttgttcatc tctgctgttc aggatcaggt cgtcccagac 180

aacactttgg cctgggtctg ggttcgtggt ttggacgaat tgtacgctga gtggagtgaa 240

gttgtgtcta caaactttcg tgatgcatca ggtccagcta tgaccgaaat tggcgaacaa 300

ccttggggcc gtgagttcgc tttacgtgat ccagccggta attgcgtgca cttcgttgct 360

gaggagcaag attag 375

<210> 112
<211> 861
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 112
atgagtattc aacatttccg tgtcgccctt attccctttt ttgcggcatt ttgccttcct 60

gtttttgctc acccagaaac gctggtgaaa gtaaaagatg ctgaagatca gttgggtgca 120

cgagtgggtt acatcgaact ggatctcaac agcggtaaga tccttgagag ttttcgcccc 180

gaagaacgtt ttccaatgat gagcactttt aaagttctgc tatgtggcgc ggtattatcc 240

cgtattgacg ccgggcaaga gcaactcggt cgccgcatac actattctca gaatgacttg 300

gttgagtact caccagtcac agaaaagcat cttacggatg gcatgacagt aagagaatta 360

tgcagtgctg ccataaccat gagtgataac actgcggcca acttacttct gacaacgatc 420

ggaggaccga aggagctaac cgcttttttg cacaacatgg gggatcatgt aactcgcctt 480

gatcgttggg aaccggagct gaatgaagcc ataccaaacg acgagcgtga caccacgatg 540

cctgtagcaa tggcaacaac gttgcgcaaa ctattaactg gcgaactact tactctagct 600

tcccggcaac aattaataga ctggatggag gcggataaag ttgcaggacc acttctgcgc 660

tcggcccttc cggctggctg gtttattgct gataaatctg gagccggtga gcgtgggtct 720

cgcggtatca ttgcagcact ggggccagat ggtaagccct cccgtatcgt agttatctac 780

acgacgggga gtcaggcaac tatggatgaa cgaaatagac agatcgctga gataggtgcc 840

tcactgatta agcattggta a 861

<210> 113
<211> 795
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 113
atgattgaac aagatggatt gcacgcaggt tctccggccg cttgggtgga gaggctattc 60

ggctatgact gggcacaaca gacaatcggc tgctctgatg ccgccgtgtt ccggctgtca 120

gcgcaggggc gcccggttct ttttgtcaag accgacctgt ccggtgccct gaatgaactg 180

caggacgagg cagcgcggct atcgtggctg gccacgacgg gcgttccttg cgcagctgtg 240

ctcgacgttg tcactgaagc gggaagggac tggctgctat tgggcgaagt gccggggcag 300

gatctcctgt catctcacct tgctcctgcc gagaaagtat ccatcatggc tgatgcaatg 360

cggcggctgc atacgcttga tccggctacc tgcccattcg accaccaagc gaaacatcgc 420

atcgagcgag cacgtactcg gatggaagcc ggtcttgtcg atcaggatga tctggacgaa 480

gagcatcagg ggctcgcgcc agccgaactg ttcgccaggc tcaaggcgcg catgcccgac 540

ggcgaggatc tcgtcgtgac ccatggcgat gcctgcttgc cgaatatcat ggtggaaaat 600

ggccgctttt ctggattcat cgactgtggc cggctgggtg tggcggaccg ctatcaggac 660

atagcgttgg ctacccgtga tattgctgaa gagcttggcg gcgaatgggc tgaccgcttc 720

ctcgtgcttt acggtatcgc cgctcccgat tcgcagcgca tcgccttcta tcgccttctt 780

gacgagttct tctga 795

<210> 114
<211> 2038
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 114
aggtggcact tttcggggaa atgtgcgcgg aacccctatt tgtttatttt tctaaataca 60

ttcaaatatg tatccgctca tgagacaata accctgataa atgcttcaat aatattgaaa 120

aaggaagagt atgagtattc aacatttccg tgtcgccctt attccctttt ttgcggcatt 180

ttgccttcct gtttttgctc acccagaaac gctggtgaaa gtaaaagatg ctgaagatca 240

gttgggtgca cgagtgggtt acatcgaact ggatctcaac agcggtaaga tccttgagag 300

ttttcgcccc gaagaacgtt ttccaatgat gagcactttt aaagttctgc tatgtggcgc 360

ggtattatcc cgtgttgacg ccgggcaaga gcaactcggt cgccgcatac actattctca 420

gaatgacttg gttgagtact caccagtcac agaaaagcat cttacggatg gcatgacagt 480

aagagaatta tgcagtgctg ccataaccat gagtgataac actgcggcca acttacttct 540

gacaacgatc ggaggaccga aggagctaac cgcttttttg cacaacatgg gggatcatgt 600

aactcgcctt gatcgttggg aaccggagct gaatgaagcc ataccaaacg acgagcgtga 660

caccacgatg cctgcagcaa tggcaacaac gttgcgcaaa ctattaactg gcgaactact 720

tactctagct tcccggcaac aattaataga ctggatggag gcggataaag ttgcaggacc 780

acttctgcgc tcggcccttc cggctggctg gtttattgct gataaatctg gagccggtga 840

gcgtgggtct cgcggtatca ttgcagcact ggggccagat ggtaagccct cccgtatcgt 900

agttatctac acgacgggga gtcaggcaac tatggatgaa cgaaatagac agatcgctga 960

gataggtgcc tcactgatta agcattggta actgtcagac caagtttact catatatact 1020

ttagattgat ttaaaacttc atttttaatt taaaaggatc taggtgaaga tccttttga 1080

taatctcatg accaaaatcc cttaacgtga gttttcgttc cactgagcgt cagaccccgt 1140

agaaaagatc aaaggatctt cttgagatcc ttttttttctg cgcgtaatct gctgcttgca 1200

aacaaaaaaa ccaccgctac cagcggtggt ttgtttgccg gatcaagagc taccaactct 1260

ttttccgaag gtaactggct tcagcagagc gcagatacca aatactgtcc ttctagtgta 1320

gccgtagtta ggccaccact tcaagaactc tgtagcaccg cctacatacc tcgctctgct 1380

aatcctgtta ccagtggctg ctgccagtgg cgataagtcg tgtcttaccg ggttggactc 1440

aagacgatag ttaccggata aggcgcagcg gtcgggctga acgggggggtt cgtgcacaca 1500

gcccagcttg gagcgaacga cctacaccga actgagatac ctacagcgtg agctatgaga 1560

aagcgccacg cttcccgaag ggagaaaggc ggacaggtat ccggtaagcg gcagggtcgg 1620

aacaggagag cgcacgaggg agcttccagg gggaaacgcc tggtatcttt atagtcctgt 1680

cgggtttcgc cacctctgac ttgagcgtcg atttttgtga tgctcgtcag gggggcggag 1740

cctatggaaa aacgccagca acgcggcctt tttacggttc ctggcctttt gctggccttt 1800

tgctcacatg ttctttcctg cgttatcccc tgattctgtg gataaccgta ttaccgcctt 1860

tgagtgagct gataccgctc gccgcagccg aacgaccgag cgcagcgagt cagtgagcga 1920

ggaagcggaa gagcgcctga tgcggtattt tctccttacg catctgtgcg gtatttcaca 1980

ccgcatatgg tgcactctca gtacaatctg ctctgatgcc gcatagttaa gccagtat 2038

<210> 115
<211> 97
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 115
gtaagtttag tctttttgtc ttttatttca ggtcccggat ccggtggtgg tgcaaatcaa 60

agaactgctc ctcagtggat gttgccttta cttctag 97

<210> 116
<211> 493
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 116
tcgaatcccg gccgggaacg gtgcattgga acgcggattc cccgtgccaa gagtgacgta 60

agtaccgcct atagagtcta taggcccaca aaaaatgctt tcttctttta atatactttt 120

ttgtttatct tatttctaat actttcccta atctctttct ttcagggcaa taatgataca 180

atgtatcatg cctctttgca ccattctaaa gaataacagt gataatttct gggttaaggc 240

aatagcaata tttctgcata taaatatttc tgcatataaa ttgtaactga tgtaagaggt 300

ttcatattgc taatagcagc tacaatccag ctaccattct gcttttattt tatggttggg 360

ataaggctgg attattctga gtccaagcta ggccctttg ctaatcatgt tcatacctct 420

tatcttcctc ccacagctcc tgggcaacgt gctggtctgt gtgctggccc atcactttgg 480

caaagaattg gga 493

<210> 117
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 117
gtaagtttag tctttttgtc ttttatttca g 31

<210> 118
<211> 56
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 118
acttaccata ctttacccgg aaactaatcg tcccactctc acatccttca ttgcag 56

<210> 119
<211> 148
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 119
aaaagttccc cagccagaag cagagaagat gatgtcaaga aatcaagggg gataaatggc 60

catagctgct gcaaatagct tattgcagtc tctagagtgt ggtaaacagg tttccagtgc 120

cagctgtgga ggtgacagcg gcagggaa 148

<210> 120
<211> 147
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 120
aaaagttccc cagccagaag cagagaagat gatgtcaaga aatcaagggg gataaatggc 60

catagctgct gcaaatagct tattgcagtc tctagagtgt ggtaaacagg tttccagtgc 120

cagctgtgga ggtgacagcg gcaggga 147

<210> 121
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 121
Ile Tyr Ile Phe Ile Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr
1 5 10 15

Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val
20 25 30

Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys
35 40 45

Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp
50 55 60

Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly
65 70 75 80

Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn
85 90 95

Tyr Leu Thr His Arg Gln Thr Asn Thr Ile
100 106