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(54) Title: SEQUENTIAL INTRAVITREAL ADMINISTRATION OF AAV GENE THERAPY TO CONTRALATERAL EYES

FIG. 4



(57) Abstract: Provided are methods of treating an ocular disease or disorder in a subject, comprising: administering a unit dose of a pharmaceutical composition to a first eye the subject via intravitreal (IVT) injection at a first time point, and administering a second unit dose of the pharmaceutical composition to a contralateral eye of the subject via IVT injection at a second time point.

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SEQUENTIAL INTRAVITREAL ADMINISTRATION OF AAV GENE THERAPY TO CONTRALATERAL EYES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application No. 62/813,597, filed March 4, 2019; and U.S. Provisional Application No. 62/839,457, filed April 26, 2019; the contents of each of which are incorporated herein by reference in their entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 627002001040SEQLIST.TXT, date recorded: March 3, 2020, size: 62 KB).

FIELD

[0003] The present disclosure relates to methods of treating ocular diseases and disorders in a subject that comprise administering a recombinant adeno associated virus (rAAV) that comprises a heterologous nucleic acid sequence encoding, e.g., aflibercept to a first eye of the subject via intravitreal (IVT) injection at a first time point, and administering the rAAV to a contralateral eye of the subject via IVT injection at a second time point.

BACKGROUND

[0004] Gene delivery vectors based on adeno-associated viruses (AAV) have demonstrated promise for the treatment of a variety of ocular diseases and disorders. However, administration of AAV-based ocular therapies remains a challenge. For example, subretinally injected AAV can efficiently transduce retinal pigment epithelium and photoreceptors in primate retina, but this mode of delivery entails puncturing the retina, which may lead to retinal tears, retinal detachment, and loss of vision. While intravitreal (IVT) AAV injection is less invasive than subretinal injection, intravitreally delivered viral vector capsid is not confined to the immune-privileged retinal compartment. Exposure of AAV capsid epitopes to the adaptive immune system in the vitreous fluid leads to the development of neutralizing antibodies ("nAb"), which may prevent effective vector re-administration. Certain ocular diseases may develop asynchronously in each eye of a patient. These asynchronous ocular diseases include wet age-related macular degeneration (wAMD), retinal vein occlusion (RVO), diabetic eye disease (DED), diabetic macular edema (DME), diabetic retinopathy (DR), choroidal neovascularization (CNV), and retinopathy of prematurity. There is, therefore, a need in the art for safe and effective methods for treating ocular diseases in each of a subject's eyes at sequential time points.

SUMMARY OF THE DISCLOSURE

[0005] Provided herein is a method of treating an ocular disease or disorder in a subject, comprising: (i) administering a first unit dose of a pharmaceutical composition to a first eye of the subject via intravitreal (IVT) injection at a first time point, and (ii) administering a second unit dose of the pharmaceutical composition to a contralateral eye of the subject via IVT injection at a second time point, wherein the pharmaceutical composition comprises: (a) a recombinant adeno-associated virus (rAAV) particle comprising a nucleic acid encoding an anti-vascular endothelial growth factor (VEGF) agent, wherein the rAAV particle is capable of infecting a retinal cell following IVT injection and (b) a pharmaceutically acceptable excipient. In some embodiments the method further comprises a step of measuring a level of neutralizing antibodies against the rAAV in a sample from the subject following the first time point and prior to the second time point. In some embodiments, the method further comprises a step of measuring expression level of the nucleic acid encoding the anti-vascular endothelial growth factor (VEGF) agent in a sample from the subject following the first time point and prior to the second time point. In some embodiments, the time interval between the first time point and the second time point is at least about 2 weeks. In some embodiments, the time interval between the first time point and the second time point at least about 4 weeks or about 1 month. In some embodiments, the time interval between the first time point and the second time point is at least about 6 weeks. In some embodiments, the time interval between the first time point and the second time point is at least about 8 weeks or about 2 months. In some embodiments, the first unit dose and the second unit dose each comprise between about $1E9$ and about $3E13$ vector genomes.

[0006] In some embodiments, the first unit dose and the second unit dose each comprise between about $1E10$ and about $1E13$ vector genomes (i.e., per eye). In some embodiments, the first unit dose and the second unit dose each comprise between about $1E11$ and about $1E13$ vector genomes (i.e., per eye). In some embodiments, the first unit dose and the second unit dose each comprise between about $2E11$ and about $6E11$ vector genomes (i.e., between about $2E11$ and about $6E11$ vector genomes per eye). In some embodiments, the first unit dose and the second unit dose each comprise between about $2E11$ and about $6E12$ vector genomes (i.e., between about $2E11$ and about $6E12$ vector genomes per eye). In some embodiments, the second unit dose is higher than the first unit dose. In some embodiments, the second unit dose is about 300% of the first unit dose (such as 3-fold or 3 times the first unit dose). In some embodiments, the second unit dose is between about 300% and about 1000% of the first unit dose. In some embodiments, the first unit dose comprises about $6E10$ vector genomes the second unit dose comprises between about $1.8E11$ and about $6E11$ vector genomes. In some embodiments, the first unit dose comprises about $6E11$ vector genomes and the second unit dose comprises between

about $1.8E12$ and about $6E12$ vector genomes. In some embodiments, wherein the first unit dose comprises about $2E11$ vector genomes and the second unit dose comprises between about $6E11$ and $2E12$ about vector genomes. In some embodiments, the first unit dose comprises about $2E12$ vector genomes and the second unit dose comprises between about $6E12$ and about $2E13$ vector genomes. In some embodiments, the volumes of first unit dose and the second unit dose are each no more than about $100\ \mu\text{L}$. In some embodiments, the volumes of first unit dose and the second unit dose are each no more than about $50\ \mu\text{L}$.

[0007] Also provided herein is a method treating an ocular disease or disorder in a subject, comprising: administering a unit dose of a pharmaceutical composition to one eye of the subject via intravitreal (IVT) injection, wherein the pharmaceutical composition comprises: (a) a recombinant adeno-associated virus (rAAV) particle comprising a nucleic acid encoding an anti-vascular endothelial growth factor (VEGF) agent, wherein the rAAV particle is capable of infecting a retinal cell following IVT injection, and (b) a pharmaceutically acceptable excipient, and wherein the subject was administered with a prior unit dose of the pharmaceutical composition to a contralateral eye via IVT injection. In some embodiments, the method further comprises a step of measuring a level of neutralizing antibodies against the rAAV in a sample from the subject following administration of the prior unit dose to the contralateral eye and prior to the administration of the unit dose to the one eye. In some embodiments, the method further comprises a step of measuring expression level of the nucleic acid encoding the anti-vascular endothelial growth factor (VEGF) agent in a sample from the subject following administration of the prior unit dose to the contralateral eye and prior to the administration of the unit dose to the one eye. In some embodiments, the unit dose comprises between about $1E10$ and about $1E13$ vector genomes. In some embodiments, the unit dose comprises between about $2E11$ and about $6E11$ vector genomes. In some embodiments, the unit dose comprises between about $2E12$ and about $6E12$ vector genomes. In some embodiments, the prior unit dose comprised between about $1E10$ and about $1E13$ vector genomes. In some embodiments, the prior unit dose comprised between about $2E11$ and about $6E11$ vector genomes. In some embodiments, the prior unit dose comprised between about $2E12$ and about $6E12$ vector genomes. In some embodiments, the unit dose administered to the one eye is higher than the prior unit dose administered to the contralateral eye. In some embodiments, the unit dose is at least about 300% of the prior unit dose. In some embodiments, the unit dose is between about 300% and about 1000% of the prior unit dose. In some embodiments, the prior unit dose comprised about $6E10$ vector genomes and the unit dose comprises between about $1.8E11$ and about $6E11$ vector genomes. In some embodiments, the prior unit dose comprised about $6E11$ vector genomes and the unit dose comprises between about $1.8E12$ and about $6E12$ vector genomes. In some embodiments, the prior unit dose comprised about $2E11$ vector genomes and the unit dose comprises between about

6E11 and 2E12 about vector genomes. In some embodiments, the prior unit dose comprised about 2E12 vector genomes and the unit dose comprises between about 6E12 and about 2E13 vector genomes. In some embodiments, the time interval between the administration of the prior unit dose and administration of the unit dose is at least about 2 weeks. In some embodiments, the time interval between the administration of the prior unit dose and administration of the unit dose is at least about 4 weeks or about 1 month. In some embodiments, the time interval between the administration of the prior unit dose and administration of the unit dose is at least about 6 weeks. In some embodiments, the time interval between the administration of the prior unit dose and administration of the unit dose is at least about 8 weeks or about two months.

[0008] In some embodiments, the rAAV particle comprises a variant capsid protein that comprises a peptide insertion relative to a corresponding parental AAV capsid protein, wherein the peptide insertion has an amino acid sequence selected from LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8), LAKDTDTTTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), and LASTGKVPNA (SEQ ID NO: 12), wherein the insertion site is located between two adjacent amino acids at a position between amino acids corresponding to amino acids 570 and 611 of VP1 of AAV2 or the corresponding position in the capsid protein of another AAV serotype. In some embodiments, the rAAV particle is an rAAV2 particle that comprises a variant capsid protein comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of SEQ ID NO: 13. In some embodiments, the variant capsid protein comprises the amino acid sequence of SEQ ID NO: 46.

[0009] In some embodiments, the rAAV particle comprises a variant capsid protein that comprises a modified sequence, the modified sequence comprising one or more amino acid substitutions within amino acid residues 570-579 relative to a parental AAV capsid protein, wherein the modified sequence comprises HKFKSGD (SEQ ID NO: 37), and wherein the amino acid residue numbering corresponds to an AAV5 VP1 capsid protein. In some embodiments, the parental AAV capsid protein is an AAV5 capsid protein or an AAV5 and AAV2 hybrid capsid protein. In some embodiments, the parental AAV capsid protein is an AAV2.5T capsid protein. In some embodiments, the parental AAV capsid protein is an AAV2.5T VP1 capsid protein. In some embodiments, the modified sequence comprises LAHKFKSGDA (SEQ ID NO: 39). In some embodiments, the variant AAV capsid protein comprises a capsid sequence having at least 85% homology to the amino acid sequence set forth in SEQ ID NO: 40 or SEQ ID NO: 41. In some embodiments, the variant AAV capsid protein comprises a capsid sequence set forth in SEQ ID NO: 42 or SEQ ID NO: 43.

[0010] In some embodiments, the anti-VEGF agent is a bevacizumab, brovacizumab, or ranibizumab. In some embodiments, the anti-VEGF agent is a polypeptide that comprises an amino acid sequence having at least 80% homology to aflibercept. In some embodiments, the anti-VEGF agent is aflibercept. In some embodiments, the retinal cell is a photoreceptor, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelium cell. In some embodiments, the ocular disease or disorder is choroidal neovascularization, wet age-related macular degeneration (wAMD), macular edema following retinal vein occlusion, diabetic macular edema (DME), or diabetic retinopathy associated with DME. In some embodiments, the ocular disease or disorder is choroidal neovascularization or wet AMD. In some embodiments, the subject is a human. In some embodiments, the subject is responsive to administration of an anti-VEGF agent, wherein the anti-VEGF agent is a polypeptide. In some embodiments, the anti-VEGF agent is aflibercept. In some embodiments, the subject received prior treatment for the ocular disease or disorder with an anti-VEGF agent. In some embodiments, the anti-VEGF agent was aflibercept.

[0011] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

INCORPORATION BY REFERENCE

[0012] All references cited herein, including patent applications and publications, are hereby incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **FIG. 1A** shows a time course of average aflibercept expression in the vitreous and aqueous fluids of the right eyes of three African green monkeys that were administered IVT with AAV2.7m8-aflibercept to the right eye on Day 0, and to the left eye on Day 59.

[0014] **FIG. 1B** shows a time course of average aflibercept expression in the vitreous and aqueous fluids of the left eyes of three African green monkeys that were administered IVT with AAV2.7m8-aflibercept to the right eye on Day 0, and to the left eye on Day 59.

[0015] **FIG. 2A** shows a time course of aflibercept expression in the vitreous fluid of the right eyes of each of the three African green monkeys that were administered IVT with AAV2.7m8-aflibercept to the right eye on Day 0, and to the left eye on Day 59.

[0016] **FIG. 2B** shows a time course of aflibercept expression in the aqueous fluid of the right eyes of each of the three African green monkeys that were administered IVT with AAV2.7m8-aflibercept to the right eye on Day 0, and to the left eye on Day 59.

[0017] **FIG. 3A** shows a time course of aflibercept expression in the vitreous fluid of the left eyes of each of the three African green monkeys that were administered IVT with AAV2.7m8-aflibercept to the right eye on Day 0, and to the left eye on Day 59.

[0018] **FIG. 3B** shows a time course of aflibercept expression in the aqueous fluid of the left eyes of each of the three African green monkeys that were administered IVT with AAV2.7m8-aflibercept to the right eye on Day 0, and to the left eye on Day 59.

[0019] **FIG. 4** shows aflibercept expression levels at study termination (*i.e.*, on Day 264) in various tissues of the right eyes and left eyes of each African green monkey that was administered IVT with AAV2.7m8-aflibercept to the right eye on Day 0 and to the left eye on Day 59.

[0020] **FIG. 5A** shows a time course of neutralizing antibody (nAb) response to 7m8 capsid protein in the vitreous liquid of the left eyes and right eyes of the three African green monkeys that were administered IVT with AAV2.7m8-aflibercept to the right eye on Day 0 and to the left eye on Day 59.

[0021] **FIG. 5B** shows a time course of neutralizing antibody (nAb) response to 7m8 capsid protein in the sera of the three African green monkeys that were administered IVT with AAV2.7m8-aflibercept to the right eye on Day 0 and to the left eye on Day 59.

[0022] **FIG. 6** provides the nucleic acid sequence of aflibercept (SEQ ID NO: 36).

[0023] **FIG. 7** provides graphs summarizing the results of monthly assessments of the levels of inflammatory keratic precipitate, vitreous cell infiltrates, aqueous haze, and aqueous cell infiltrates in the right eyes (*i.e.*, first eyes) and left eyes (*i.e.*, later dosed eyes) of monkeys who were administered IVT with AAV2.7m8-aflibercept as compared to a monkey that received IVT vehicle injection. Arrows with dotted lines indicate time of injection of AAV2.7m8-aflibercept.

[0024] **FIG. 8** provides the results of optical coherence tomography measurements taken monthly to determine the effects of staggered bilateral dosing of AAV2.7m8-aflibercept on retinal thickness and retinal volume.

[0025] **FIG. 9** provides sections of retinal tissue that were stained with haematoxylin and eosin in order to assess retinal morphology, including cell death and immune cell infiltration.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0026] The methods, compositions, and kits 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.

Definitions

[0027] Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

[0028] The terminology used herein is for the purpose of describing particular examples only and is not intended to be limiting. 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". The term "comprising" as used herein is synonymous with "including" or "containing", and is inclusive or open-ended.

[0029] Any reference to "or" herein is intended to encompass "and/or" unless otherwise stated. As used herein, the term "about" a number refers to that number plus or minus 10% of that number. The term "about" a range refers to that range minus 10% of its lowest value and plus 10% of its greatest value.

[0030] The term "subject", "patient", or "individual" refers to primates, such as humans and non-human primates, e.g., African green monkeys and rhesus monkeys. In some embodiments, the subject is a human.

[0031] The terms "treat," "treating", "treatment," "ameliorate" or "ameliorating" and other grammatical equivalents as used herein, refer to alleviating, abating or ameliorating an ocular disease or disorder or symptoms of the ocular disease or disorder, preventing additional symptoms of the

ocular disease or disorder, ameliorating or preventing the underlying metabolic causes of symptoms, inhibiting the ocular disease or disorder, e.g., arresting the development of the ocular disease or disorder, relieving the ocular disease or disorder, causing regression of the ocular disease or disorder, or stopping the symptoms of the ocular disease or disorder, and are intended to include prophylaxis. The terms further include achieving a therapeutic benefit and/or a prophylactic benefit. The term “therapeutic benefit” refers to eradication or amelioration of the ocular disease or disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the ocular disease or disorder such that an improvement is observed in the patient, notwithstanding that, in some embodiments, the patient is still afflicted with the ocular disease or disorder. For prophylactic benefit, the pharmaceutical compositions are administered to a patient at risk of developing the ocular disease or disorder, or to a patient reporting one or more of the physiological symptoms of the ocular disease or disorder, even if a diagnosis of the disease or disorder has not been made. Patients with asynchronous disease development may receive therapeutic benefit from treatment of their eye with more advanced disease, and prophylactic benefit from treatment of their eye with less advanced disease.

[0032] The terms “administer,” “administering,” “administration,” and the like, as used herein, can refer to the methods that are used to enable delivery of therapeutics or pharmaceutical compositions to the desired site of biological action. These methods include intravitreal or subretinal injection to an eye.

[0033] The terms “effective amount”, “therapeutically effective amount” or “pharmaceutically effective amount” as used herein, can refer to a sufficient amount of at least one pharmaceutical composition or compound being administered which will relieve to some extent one or more of the symptoms of the ocular disease or disorder being treated. An “effective amount”, “therapeutically effective amount” or “pharmaceutically effective amount” of a pharmaceutical composition may be administered to a subject in need therein if as a unit dose (as described in further detail elsewhere herein).

[0034] The term “pharmaceutically acceptable” as used herein, can refer to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of a compound disclosed herein, and is relatively nontoxic (i.e., when the material is administered to an individual it does not cause undesirable biological effects nor does it interact in a deleterious manner with any of the components of the composition in which it is contained).

[0035] The term “pharmaceutical composition,” or simply “composition” as used herein, can refer to a biologically active compound, optionally mixed with at least one pharmaceutically acceptable chemical component, such as, though not limited to carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, excipients and the like.

[0036] An “AAV vector” or “rAAV vector” as used herein refers to an adeno-associated virus (AAV) vector or a recombinant AAV (rAAV) vector comprising a polynucleotide sequence not of AAV origin (e.g., a polynucleotide heterologous to AAV such as a nucleic acid sequence that encodes a therapeutic transgene, e.g., aflibercept) for transduction into a target cell or to a target tissue. In general, the heterologous polynucleotide is flanked by at least one, and generally by two, AAV inverted terminal repeat sequences (ITRs). The term rAAV vector encompasses both rAAV vector particles and rAAV vector plasmids. A rAAV vector may either be single-stranded (ssAAV) or self-complementary (scAAV).

[0037] An “AAV virus” or “AAV viral particle” or “rAAV vector particle” or “rAAV particle” refers to a viral particle comprising at least one AAV capsid protein (typically by all of the capsid proteins of a wild-type AAV) and a polynucleotide rAAV vector. If the particle comprises a heterologous polynucleotide (e.g., a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a target cell or target tissue), it is typically referred to as an “rAAV vector particle” or an “rAAV vector”. Thus, production of rAAV particle necessarily includes production of an rAAV vector, as such a vector contained within an rAAV particle.

[0038] The term “packaging” as used herein can refer to a series of intracellular events that can result in the assembly and encapsidation of a rAAV particle.

[0039] AAV “rep” and “cap” genes refer to polynucleotide sequences encoding replication and encapsidation proteins of adeno-associated virus. AAV rep and cap are referred to herein as AAV “packaging genes.”

[0040] The term “polypeptide” can encompass both naturally-occurring and non-naturally occurring proteins (e.g., a fusion protein), peptides, fragments, mutants, derivatives and analogs thereof. A polypeptide may be monomeric, dimeric, trimeric, or polymeric. Further, a polypeptide may comprise a number of different domains each of which has one or more distinct activities. For the avoidance of doubt, a “polypeptide” may be any length greater two amino acids.

[0041] As used herein, “polypeptide variant” or simply “variant” refers to a polypeptide whose sequence contains an amino acid modification. In some embodiments, the modification is an insertion, duplication, deletion, rearrangement or substitution of one or more amino acids compared to the amino acid sequence of a reference protein or polypeptide, such as a native or wild-type protein. A variant may have one or more amino acid point substitutions, in which a single amino acid at a position has been changed to another amino acid, one or more insertions and/or deletions, in which one or more amino acids are inserted or deleted, respectively, in the sequence of the reference protein, and/or truncations of the amino acid sequence at either or both the amino or carboxy termini. A variant can have the same or a different biological activity compared to the reference protein, or the unmodified protein.

[0042] In some embodiments, a variant can have, for example, at least about any one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% overall sequence homology to its counterpart reference protein. In some embodiments, a variant can have at least about 90% overall sequence homology to the wild-type protein. In some embodiments, a variant exhibits at least about 95%, at least about 98%, at least about 99%, at least about 99.5%, or at least about 99.9% overall sequence identity.

[0043] As used herein, “recombinant” can refer to a biomolecule, e.g., a gene or protein, that (1) has been removed from its naturally occurring environment, (2) is not associated with all or a portion of a polynucleotide in which the gene is found in nature, (3) is operatively linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature. The term “recombinant” can be used in reference to cloned DNA isolates, chemically synthesized polynucleotide analogs, or polynucleotide analogs that are biologically synthesized by heterologous systems, as well as proteins and/or mRNAs encoded by such nucleic acids. Thus, for example, a protein synthesized by a microorganism is recombinant, for example, if it is synthesized from an mRNA synthesized from a recombinant gene present in the cell.

[0044] The term “anti-VEGF agent” includes any therapeutic agent, including proteins, polypeptides, peptides, fusion protein, multimeric proteins, gene products, antibody, human monoclonal antibody, antibody fragment, aptamer, small molecule, kinase inhibitor, receptor or receptor fragment, or nucleic acid molecule, that can reduce, interfere with, disrupt, block and/or inhibit the activity or function of an endogenous VEGF and/or an endogenous VEGF receptor (VEGFR), or the VEGF-VEGFR interaction or pathway in vivo. An anti-VEGF agent can be any one of the known therapeutic agents that can reduce new blood vessel growth or formation and/or edema, or swelling, when delivered into a cell, tissue, or a subject in vivo, e.g., ranibizumab, brolucizumab, or bevacizumab. In some embodiments, an anti-VEGF agent can be naturally occurring, non-naturally occurring, or synthetic. In some embodiments, an anti-VEGF agent can be derived from a naturally occurring molecule that was subsequently modified or mutated to confer an anti- VEGF activity. In some embodiments, an anti-VEGF agent is a fusion or chimeric protein. In such proteins, functional domains or polypeptides are artificially fused to a moiety or a polypeptide to make a fusion or chimeric protein that can sequester VEGF in vivo or function as a VEGFR decoy. In some embodiments, an anti-VEGF agent is a fusion or chimeric protein that blocks endogenous VEGFR from interacting with its ligands.

[0045] As used herein, “VEGF” can refer to any isoform of VEGF, unless required otherwise, including, but not limited to, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, or any combination, or any functional fragment or variant thereof. Unless required otherwise, “VEGF” can refer to any member of the VEGF family, including members: VEGF-A, placenta growth factor (PGF), VEGF-B, VEGF-C, and VEGF-D, or any combination, functional fragment, or variant thereof.

As used herein, “VEGF receptor” or “VEGFR” or “VEGF-R” can be used to refer to any one of the receptors of VEGF, including, but not limited to, VEGFR-1 (or Flt-1), VEGFR-2 (or Flk-1/KDR), and VEGFR-3 (or Flt-4). VEGFR can be a membrane bound or soluble form, or a functional fragment or truncation of a receptor. Examples of anti-VEGF agent include, but are not limited to, ranibizumab, bevacizumab, brolucizumab, or any combination, variant, or functional fragment thereof.

[0046] “Operatively linked” or “operably linked” or “coupled” can refer to a juxtaposition of genetic elements, wherein the elements are in a relationship permitting them to operate in an expected manner. For instance, a promoter can be operatively linked to a coding region if the promoter helps initiate transcription of the coding sequence. There may be intervening residues between the promoter and coding region so long as this functional relationship is maintained.+++

[0047] The term “expression vector” or “expression construct” or “cassette” or “plasmid” or simply “vector” can include any type of genetic construct, including AAV or rAAV vectors, containing a nucleic acid or polynucleotide coding for a gene product in which part or all of the nucleic acid encoding sequence is capable of being transcribed and is adapted for gene therapy. The transcript can be translated into a protein. In some embodiments, the transcript is 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. An expression vector can also comprise control elements operatively linked to the encoding region to facilitate expression of the protein in target cells. The combination of control elements and a gene or genes to which they are operably linked for expression can sometimes be referred to as an “expression cassette,” a large number of which are known and available in the art or can be readily constructed from components that are available in the art.

[0048] The term “heterologous” can refer to an entity that is genotypically distinct from that of the rest of the entity to which it is being compared. For example, a polynucleotide introduced by genetic engineering techniques into a plasmid or vector derived from a different species can be a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence with which it is not naturally found linked can be a heterologous promoter.

[0049] As used herein, “7m8” refers to the amino acid sequence LALGETTRPA (SEQ ID NO: 1).

[0050] “7m8 variant” refers to a rAAV, which can be of any serotype, with the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted in the solvent exposed GH loop of the capsid protein.

[0051] When 7m8 is inserted in a rAAV2 (also referred to as AAV2.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop of the AAV2 capsid protein,

e.g., between positions 587 and 588 of the AAV2 capsid protein, VP1. When 7m8 is inserted in a rAAV1 (also referred to as AAV1.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop of the AAV1 capsid protein, e.g., between amino acids 590 and 591 of the AAV1 capsid protein. When 7m8 is inserted in a rAAV5 (also referred to as AAV5.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop of the AAV5 capsid protein, e.g., between amino acids 575 and 576 of the AAV5 capsid protein. When 7m8 is inserted in a rAAV6 (also referred to as AAV6.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop of the AAV6 capsid protein, e.g., between amino acids 590 and 591 of the AAV6 capsid protein. When 7m8 is inserted in a rAAV7 (also referred to as AAV7.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop of the AAV7 capsid protein, e.g., between amino acids 589 and 590 of the AAV7 capsid protein. When 7m8 is inserted in a rAAV8 (also referred to as AAV8.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop of the AAV8 capsid protein, e.g., between amino acids 590 and 591 of the AAV8 capsid protein. When 7m8 is inserted in a rAAV9 (also referred to as AAV9.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop of the AAV9 capsid protein, e.g., between amino acids 588 and 589 of the AAV9 capsid protein. When 7m8 is inserted in a rAAV10 (also referred to as AAV10.7m8), the amino acid sequence LALGETTRPA (SEQ ID NO: 1) is inserted into the GH loop of the AAV10 capsid protein, e.g., between amino acids 589 and 590 of the AAV10 capsid protein.

Overview

[0052] The ability of AAV vectors (*e.g.*, AAV2.7m8) to efficiently transduce target retinal cells following IVT injection has been exploited to successfully transfer therapeutic genes into photoreceptors, retinal pigment epithelium, and the inner retina to treat a variety of retinal diseases. Intravitreal (IVT) AAV administration is a safe and convenient method of retinal delivery, but it has been suggested that neutralizing antibodies (nAb) against the vector capsid are more likely to be generated following IVT injection than following subretinal injection. Given that certain ocular diseases, such as wet age-related macular degeneration (wAMD), can develop asynchronously in both of an individual's eyes, there is a concern that nAb generated following IVT administration of an AAV to a first eye may decrease the efficiency of therapeutic gene transfer and prevent effective vector re-administration, *e.g.*, to the individual's contralateral eye. The methods provided herein are based on Applicant's finding that that development of immunity (*e.g.*, neutralizing antibodies or "nAb") following IVT administration of an rAAV2-based vector to a subject's first eye does not completely block transduction following administration of the vector via IVT injection to the subject's contralateral eye.

Methods of Treatment

[0053] Provided herein is a method of treating, slowing the progression of, and/or preventing an ocular disease or disorder in a subject that comprises (i) administering a first unit dose of a pharmaceutical composition to a first eye of the subject via intravitreal (IVT) injection at a first time point, and (ii) administering a second unit dose of the pharmaceutical composition to a contralateral eye of the subject via IVT injection at a second time point, wherein the pharmaceutical composition comprises: (a) a recombinant adeno-associated virus (rAAV) particle comprising a nucleic acid encoding an anti-vascular endothelial growth factor (VEGF) agent, wherein the rAAV particle is capable of infecting a retinal cell following IVT injection and (b) a pharmaceutically acceptable excipient.

[0054] In some embodiments, the method further comprises a step of measuring a level of neutralizing antibodies (nAbs) against the rAAV in a sample from the subject following the first time point and prior to the second time point. In some embodiments, the sample is a serum sample, a vitreous fluid sample, or an aqueous fluid sample. In some embodiments, measuring the level of nAbs comprises measuring the potency of neutralizing antibodies (nAbs) against the rAAV. In some embodiments, the neutralizing potency is quantified by the inhibitory concentration (IC), defined as the concentration of nAb (e.g., serum nAb) at which rAAV infectivity has been reduced by 50% relative to the absence of nAb (i.e., IC₅₀). In some embodiments, rAAV particle infectivity is measured in an *in vitro* cell-based assay in a cell type that is readily transduced by the rAAV, for example HEK293T cells. In some embodiments IC₅₀ is expressed as the dilution factor necessary for a nAb-containing sample to cause a 50% reduction in rAAV particle infectivity. For example, where a nAb-containing sample required a 1:500 dilution to cause a 50% reduction in rAAV infectivity, the IC₅₀ may be expressed as 500. In some embodiments, the IC₅₀ is less than about any one of 600, 550, 500, 450, 400, 350, 300, 250, 200, 125, or 100. In some embodiments, the time interval between the first time point and the second time point is at least about any one of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks, at least about any one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 15, or 20 years, including any range between these values, and the step of measuring the level of neutralizing antibodies (nAbs) against the rAAV in the sample from the subject occurs within the time interval.

[0055] In some embodiments, the step of measuring the levels of nAbs against the rAAV in a sample from the subject following the first time point and prior to the second time point is accomplished by means of a cut-point nAb assay. In a cut-point assay, the level of inhibition of rAAV infectivity caused by a test sample is compared to a predetermined cut point, above which the sample is determined to be positive for nAbs, and below which the sample is determined to be negative. The cut point is set at the level below which a predetermined percentage (e.g. 95%) of test samples taken from an rAAV-naïve population fall.

[0056] In some embodiments, the method further comprises a step of measuring expression level of the nucleic acid encoding the anti-VEGF agent in a sample from the subject following the first time point and prior to the second time point. In some embodiments, the sample is a vitreous fluid sample or an aqueous fluid sample. In some embodiments, the expression level of the nucleic acid is measured by determining the abundance (e.g., relative abundance) of mRNA encoding the anti-VEGF agent. In some embodiments, the abundance (e.g., relative abundance) of mRNA is determined (e.g., quantified) via northern blot, RT-qPCR, RNA sequencing, RNA in situ hybridization, or other methods known in the art. In some embodiments, the expression level of the nucleic acid is measured by determining the abundance (e.g., relative abundance) of the anti-VEGF agent encoded by the nucleic acid. In some embodiments, the abundance (e.g., relative abundance) of anti-VEGF agent is determined (e.g., quantified) via western blot, Liquid chromatography–mass spectrometry (LC/MS), ELISA, immunohistochemistry, or other immunoassays known in the art. In some embodiments, the time interval between the first time point and the second time point is at least about any one of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks; at least about any one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 15, or 20 years, including any range between these values, and the step of measuring the expression level of the nucleic acid encoding the anti-VEGF agent in a sample from the subject occurs within the time interval.

[0057] In some embodiments the first and second unit doses are each therapeutically effective doses, e.g., doses sufficient to ensure efficient delivery of the nucleic acid (e.g., the nucleic acid encoding an anti-VEGF agent, such as aflibercept) into target cells (such as retinal) cells. In some embodiments, the first unit dose and the second unit dose are the same, e.g., between about 1E9 to about 3E13 vector genomes, between about 1E10 and about 1E13 vector genomes, between about 1E11 and 1E13 vector genomes, between about 1E10 to about 3E12 vector genomes, or between about 2E12 and about 6E12 vector genomes. In some embodiments, the second unit dose is higher than the first unit dose, e.g., at least about any one of 150%, 175%, 200%, 225%, 250%, 275%, or 300%, 350%, 400%, 450%, 500%, 550%, 600%, 650%, 700%, 750%, 800%, 850%, 900%, 950%, or 1000% of the first unit dose, including any range in between these values. For example, in some embodiments, the second unit dose is at least about any one of 1.5-fold, 1.75-fold, 2-fold, 2.25-fold, 2.5-fold, 2.75-fold, 3-fold, 3.25-fold, 3.5-fold, 4-fold, 4.5-fold, 5-fold, 5.5-fold, 6-fold, 6.5-fold, 7-fold, 7.5-fold, 8-fold, 8.5-fold, 9-fold, 9.5-fold, or 10-fold of the first unit dose, including any range in between these values. In some embodiments, the first unit dose comprises about 6E10 vector genomes and the second unit dose comprises between about 1.8E11 and about 6E11 vector genomes. In some embodiments, the second unit dose comprises about 1.8E11 vector genomes or about 6E11 vector genomes. In some embodiments, the first unit dose comprises about 2E11 vector genomes and the second unit dose comprises between about 6E11 and about 2E12

vector genomes. In some embodiments, the second unit dose comprises about 6E11 vector genomes or about 2E12 vector genomes. Additional details regarding unit doses are provided elsewhere herein. In some embodiments, the volume of the first unit dose is no more than about any one of 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15 or 10 μ L. In some embodiments, the volume of the second unit dose is no more than about any one of 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15 or 10 μ L.

[0058] Also provided is a method treating an ocular disease or disorder in a subject, comprising: administering a unit dose of a pharmaceutical composition to one eye of the subject via intravitreal (IVT) injection, wherein the pharmaceutical composition comprises: (a) a recombinant adeno-associated virus (rAAV) particle comprising a nucleic acid encoding a therapeutic protein, for example, an anti-vascular endothelial growth factor (VEGF) agent, wherein the rAAV particle is capable of infecting a retinal cell following IVT injection, and (b) a pharmaceutically acceptable excipient, and wherein the subject was administered with a prior unit dose of the pharmaceutical composition to a contralateral eye via IVT injection.

[0059] In some embodiments, the method further comprises a step of measuring a level of neutralizing antibodies against the rAAV in a sample from the subject following administration of the pharmaceutical composition to the contralateral eye and prior to the administration of pharmaceutical composition to the one eye. In some embodiments, the sample is a serum sample, a vitreous fluid sample, or an aqueous fluid sample. In some embodiments, measuring the level of nAbs comprises measuring the potency of neutralizing antibodies (nAbs) against the rAAV. In some embodiments, the neutralizing potency is quantified by the inhibitory concentration (IC), defined as the concentration of nAb (e.g., serum nAb) at which rAAV infectivity has been reduced by 50% relative to the absence of nAb (i.e., IC₅₀). In some embodiments, rAAV particle infectivity is measured in an *in vitro* HEK293T cell-based assay. In some embodiments IC₅₀ is expressed as the dilution factor necessary for a nAb-containing sample to cause a 50% reduction in rAAV particle infectivity. For example, where a nAb-containing sample required a 1:500 dilution to cause a 50% reduction in rAAV infectivity, the IC₅₀ may be expressed as 500. In some embodiments, the IC₅₀ is less than about any one of 600, 550, 500, 450, 400, 350, 300, 250, or 200. In some embodiments, the time interval between the step of administering the prior unit dose of the pharmaceutical composition to the contralateral eye and the step of administering the unit dose of the pharmaceutical composition to the one eye is at least about any one of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks, at least about any one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 15, or 20 years, including any range between these values, and the step of measuring the level of neutralizing antibodies (nAbs) against the rAAV in the sample from the subject occurs within the time interval. In some embodiments, the level of

neutralizing antibodies against the rAAV in a sample from the subject is measured via cut-point nAb assay, which is described elsewhere herein.

[0060] In some embodiments, the method further comprises a step of measuring expression level of the nucleic acid encoding the anti-VEGF agent in a sample from the subject following administration of the pharmaceutical composition to the contralateral eye and prior to the administration of pharmaceutical composition to the one eye. In some embodiments, the sample is a vitreous fluid sample or an aqueous fluid sample. In some embodiments, the expression level of the nucleic acid is measured by determining the abundance (e.g., relative abundance) of mRNA encoding the anti-VEGF agent. In some embodiments, the abundance (e.g., relative abundance) of mRNA is determined (e.g., quantified) via northern blot, RT-qPCR, RNA sequencing, RNA in situ hybridization, or other methods known in the art. In some embodiments, the expression level of the nucleic acid is measured by determining the abundance (e.g., relative abundance) of the anti-VEGF agent encoded by the nucleic acid. In some embodiments, the abundance (e.g., relative abundance) of anti-VEGF agent is determined (e.g., quantified) via western blot, Liquid chromatography–mass spectrometry (LC/MS), ELISA, immunohistochemistry, or other immunoassays known in the art. In some embodiments, the time interval between the step of administering the prior unit dose of the pharmaceutical composition to the contralateral eye and the step of administering the unit dose of the pharmaceutical composition to the one eye is at least about any one of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks; at least about any one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months; or at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 15, or 20 years, including any range between these values, and the step of measuring the expression level of the nucleic acid encoding the anti-VEGF agent in a sample from the subject occurs within the time interval.

[0061] In some embodiments the unit dose is a therapeutically effective dose, e.g., a dose sufficient to ensure efficient delivery of the nucleic acid (e.g., the nucleic acid encoding an anti-VEGF agent) into target cells (such as retinal) cells. In some embodiments, the prior unit dose was a therapeutically effective dose. In some embodiments, the unit dose and the prior unit dose are the same, e.g., between about 1E9 to about 3E13 vector genomes, between about 1E10 and about 1E13 vector genomes, between about 1E11 and 1E13 vector genomes, between about 1E10 to about 3E12 vector genomes, or between about 2E12 and about 6E12 vector genomes. In some embodiments, the unit dose is higher than the prior unit dose, e.g., at least about any one of 150%, 175%, 200%, 225%, 250%, 275%, or 300%, 350%, 400%, 450%, 500%, 550%, 600%, 650%, 700%, 750%, 800%, 850%, 900%, 950%, or 1000% of the prior unit dose, including any range in between these values. For example, in some embodiments, the unit dose is at least about any one of 1.5-fold, 1.75-fold, 2-fold, 2.25-fold, 2.5-fold, 2.75-fold, 3-fold, 3.25-fold, 3.5-fold, 4-fold, 4.5-fold, 5-fold, 5.5-fold, 6-fold, 6.5-fold, 7-fold, 7.5-fold, 8-fold, 8.5-fold, 9-fold, 9.5-fold,

or 10-fold of the prior unit dose, including any range in between these values. In some embodiments, the prior unit dose comprised about 6E10 vector genomes and the unit dose comprises between about 1.8E11 and about 6E11 vector genomes. In some embodiments, the unit dose comprises about 1.8E11 vector genomes or about 6E11 vector genomes. In some embodiments, the prior unit dose comprised about 2E11 vector genomes and the unit dose comprises between about 6E11 and about 2E12 vector genomes. In some embodiments, the unit dose comprises about 6E11 vector genomes or about 2E12 vector genomes. Additional details regarding unit doses are provided elsewhere herein. In some embodiments, the volume of the unit dose is no more than about any one of 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15 or 10 μ L. In some embodiments, the volume of the prior unit dose was no more than about any one of 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15 or 10 μ L.

[0062] In some embodiments, the rAAV particle administered to the subject to treat and ocular disease or disorder comprises a variant capsid protein, wherein the variant capsid protein comprises an insertion of a peptide in the capsid protein GH loop relative to a corresponding parental capsid protein, wherein the insertion comprises an amino acid sequence selected from LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8), LAKDTDTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), and LASTGKVPNA (SEQ ID NO: 12). In some embodiments, the insertion site is within amino acids 570-611 of the AAV2 capsid protein set forth in SEQ ID NO: 13, or the corresponding position in the capsid protein of another AAV serotype. In some embodiments, the rAAV particle administered to the subject to treat and ocular disease or disorder is an rAAV2 particle comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of SEQ ID NO: 13. The amino acid sequence of SEQ ID NO: 13 is provided below:

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MAADGYLPDW LEDTLSEGIR QWWKLKPGPP PPKPAERHKD DSRGLVLPGY KYLGPFNGLD
KGEFVNEADA AALEHDKAYD RQLDSGDNPY LKYNHADA EF QERLKEDTSF GGNLGRAVFQ
AKKRVLEPLG LVEEPVK TAP GKKRPVEHSP VEPDSSSGTG KAGQQPARKR LNFGQTGDAD
SVPDPQPLGQ PPAAPSGLT NTMATGSGAP MADNNEGADG VGNSSGNWHC DSTWMGDRVI
TTSTRTWALP TYNNHLYKQI SSQSGASNDN HYFGYSTPWG YFDFNRFHCH FSPRDWQRLI
NNNWGFRPKR LNFKLFNIQV KEVTQNDGTT TIANNLTSTV QVFTDSEYQL PYVLGSAHQG
CLPPFPADV F MVPQYGYLTL NNGSQAVGRS SFYCLEYFPS QMLRTGNNFT FSYTFEDVPF
HSSYAHSQSL DRLMNPLIDQ YLYYLSRTNT PSGTTTQSRL QFSQAGASDI RDQSRNWLPG
PCYRQQRVSK TSADNNNSEY SWTGATKYHL NGRDSLVPNG PAMASHKDDE EKFFPQSGVL
IFGKQGSEKT NVDIEKVMIT DEEEIRTTNP VATEQYGSVS TNLQRGNRQA ATADVNTQGV
LPGMVWQDRD VYLQGPWAK IPHTDGHFHP SPLMGGFGLK HPPPQILIKN TPVPANPSTT
FSAAKFASFI TQYSTGQVSV EIEWELQKEN SKRWNPEIQY TSNYNKSNNV DFTVDITNGVY
SEPRPIGTRY LTRNL (SEQ ID NO: 13)

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[0063] In some embodiments, the rAAV particle administered to the subject to treat and ocular disease or disorder is an rAAV2 particle comprising the amino acid sequence of SEQ ID NO: 46, which is provided below:

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MAADGYLPDW LEDTLSEGIR QWWKLKPGPP PPKPAERHKD DSRGLVLPGY KYLGPFNGLD
KGEVPNEADA AALEHDKAYD RQLDSGDNPY LKYNHADA EF QERLKEDTSF GGNLGRAVFQ
AKKRVLEPLG LVEEPVKTAP GKKRPVEHSP VEPDSSSGTG KAGQQPARKR LNFGQTGDAD
SVPDPQPLGQ PPAAPSGLGT NTMATGSGAP MADNNEGADG VGNSSGNWHC DSTWMGDRVI
TTSTRTWALP TYNNHLYKQI SSQSGASNDN HYFGYSTPWG YFDENRFHCH FSPRDWQRLI
NNNWGFRPKR LNFKLFNIQV KEVTQNDGTT TIANNLTSTV QVFTDSEYQL PYVLGSAHQG
CLPPFPADV F MVPQYGYLTL NNGSQAVGRS SFYCLEYFPS QMLRTGNNFT FSYTFEDVFPF
HSSYAHSQSL DRLMNPLIDQ YLYYLSRTNT PSGTTTQSRL QFSQAGASDI RDQSRNWLPG
PCYRQQRVSK TSADNNNSEY SWTGATKYHL NGRDSLVPNG PAMASHKDDE EKFFFPQSGVL
IFGKQGSEKT NVDIEKVMIT DEEEIRTTNP VATEQYGSVS TNLQRGNLAL GETTRPARQA
ATADVNTQGV LPGMVWQDRD VYLQGPWAK IPHTDGHFHP SPLMGGFGLK HPPQILIKN
TPVPANPSTT FSAAKFASFI TQYSTGQVSV EIEWELQKEN SKRWNPEIQY TSNYNKSVNV
DFTVDTNGVY SEPRPIGTRY LTRNL (SEQ ID NO: 46)

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[0064] In some embodiments, the rAAV particle administered to the subject to treat and ocular disease or disorder comprises a variant capsid protein, wherein the variant capsid protein comprises a peptide insertion relative to a corresponding parental AAV capsid protein, wherein the peptide insertion has an amino acid sequence selected from LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8), LAKDTDTTTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), and LASTGKVPNA (SEQ ID NO: 12), wherein the insertion site is located between two adjacent amino acids at a position between amino acids corresponding to amino acids 570 and 611 of VP1 of AAV2 or the corresponding position in the capsid protein of another AAV serotype.

[0065] In some embodiments, rAAV particle administered to the subject to treat and ocular disease or disorder comprises a variant capsid protein, wherein the variant capsid protein comprises a modified sequence comprising one or more amino acid substitutions within amino acid residues 570-579 relative to a parental AAV capsid protein, wherein the modified sequence comprises HKFKSGD (SEQ ID NO: 1), and wherein the amino acid residue numbering corresponds to an AAV5 VP1 capsid protein. In some embodiments, the parental AAV capsid protein is an AAV5 capsid protein or an AAV5 and AAV2 hybrid capsid protein. In some embodiments, the parental AAV capsid protein is an AAV2.5T capsid protein. In some embodiments, “AAV2.5T capsid protein” or “AAV2.5T variant” refers to a hybrid capsid containing regions from AAV2 and AAV5, described in U.S. Patent No. 9,441,244, the disclosure of which is incorporated in its entirety. AAV2.5T is capable of transducing the retina when delivered subretinally, but not when injected intravitreally. AAV2.5T transduction may be blocked by the inner limiting membrane (ILM), which is enriched

with heparin sulfate proteoglycan (HSPG). The surface-exposed domains of AAV2.5T are identical to that of AAV5 except for a single substitution of A to T in aa582 of AAV2.5T (aa581 of AAV5), a mutation which appears to increase infectivity in mammalian cells without impacting AAV5's typical sialic acid receptor binding. AAV5 and AAV2.5T have negligible heparin sulfate binding, whereas AAV2 has high affinity for heparin sulfate. In some embodiments, the parental AAV capsid protein is an AAV2.5T VP1 capsid protein. In some embodiments, the modified sequence comprises LAHKFKSGDA (SEQ ID NO: 3). In some embodiments, the rAAV is AAV2.5T.LSV. In some embodiments, "AAV2.5T.LSV" or "AAV2.5T.LSV variant" refers to a rAAV variant that comprises a variant capsid protein, wherein the variant capsid protein comprises a loop substitution variant, wherein the loop substitution variant comprises the amino acid loop sequence LAHKFKSGDA (SEQ ID NO: 3) at amino acid residues 570-579 relative to AAV2.5T, the parental AAV capsid protein. Further details regarding recombinant adeno-associated viral vectors that can be used with the methods of the present application are provided elsewhere herein

[0066] In some embodiments, the anti-VEGF agent is a bevacizumab, brotuzumab, or ranibizumab. In some embodiments, the anti-VEGF agent is a polypeptide that comprises an amino acid sequence having at least 80% homology to aflibercept. In some embodiments, the anti-VEGF agent is aflibercept.

[0067] In some embodiments, the retinal cell is a photoreceptor, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelium cell.

[0068] In some embodiments, the ocular disease or disorder is characterized by abnormal (e.g., excessive) angiogenesis or neovascularization. In some embodiments, the ocular disease or disorder is, e.g., choroidal neovascularization, neovascular (wet) age-related macular degeneration (wAMD), macular edema following retinal vein occlusion, diabetic macular edema (DME), or diabetic retinopathy associated with DME, retinal vein occlusion, or any other related ocular disease or disorder characterized by abnormal (e.g., excessive) neovascularization in a subject. In some embodiments, ocular disease or disorder is a disease or disorder that is responsive to treatment with aflibercept (EYLEA®). In some embodiments, methods described herein are used to treat an ocular disease or disorder that is responsive to the current standard of care or is responsive to at least one of the approved therapies for AMD, RVO, DME, or DR in patients with DME, such as aflibercept injection, ranibizumab injection, brotuzumab injection, or bevacizumab injection.

[0069] In some embodiments, the pharmaceutical composition comprises a vector capable of delivering a nucleic acid encoding a polypeptide comprising an amino acid sequence that has at least 80% homology (such as at least any one of about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% homology) to aflibercept to retinal cells. In some embodiments, the pharmaceutical composition comprises a recombinant adeno-associated viral vector rAAV2 variant comprising a

variant capsid protein that comprises amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of capsid protein VP1 (e.g., as set forth in SEQ ID NO: 13), and a nucleic acid encoding a polypeptide comprising an amino acid sequence that has at least 80% homology (such as at least any one of about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% homology) to aflibercept and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition comprises a recombinant adeno-associated viral vector rAAV2 variant comprising a variant capsid protein that comprises the amino acid sequence of SEQ ID NO: 46, and a nucleic acid encoding a polypeptide comprising an amino acid sequence that has at least 80% homology (such as at least any one of about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% homology) to aflibercept and a pharmaceutically acceptable excipient. In some embodiments, the nucleic acid encodes aflibercept.

[0070] In some embodiments, the pharmaceutical composition comprises a recombinant adeno-associated viral vector rAAV2.5T variant comprising a variant capsid protein that comprises a loop substitution variant, wherein the loop substitution variant comprises the amino acid loop sequence LAHKFKSGDA (SEQ ID NO: 3) at amino acid residues 570-579 relative to AAV2.5T, the parental AAV capsid protein, and a nucleic acid encoding a polypeptide comprising an amino acid sequence that has at least 80% homology (such as at least any one of about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% homology) to aflibercept and a pharmaceutically acceptable excipient. In some embodiments, the nucleic acid encodes aflibercept. The protein sequence of aflibercept is publicly available at DrugBank database, accession number DB08885. In some embodiments, aflibercept refers to a nucleic acid sequence that encodes the protein, as disclosed in U.S. Patent Pub. 2014/0371438 (*see, e.g., FIG. 6*).

[0071] In some embodiments, the aflibercept (or functional fragment thereof or functional variant thereof) encoded by the nucleic acid delivered by the vector (e.g., recombinant adeno-associated viral vector) is expressed at a therapeutic dose in the target cells (e.g., retinal cells) of the first eye and the contralateral eye. In some embodiments, the aflibercept is expressed at a therapeutic dose in the first eye and the contralateral eye for at least about any one of 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or for more than 12 months, e.g., at least about 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 15, or 20 years, including any range between any of these values, following administration via IVT injection to the first eye. In some embodiments, the aflibercept is expressed at a therapeutic dose in the first eye and the contralateral eye for at least about any one of 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or for more than 12 months, e.g., at least about any one of 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 15, or 20 years, including any range between any of these values, following administration via IVT injection to the contralateral eye.

[0072] In some embodiments, the subject is a human or a non-human primate (e.g., an African green monkey or a rhesus macaque). In some embodiments, the subject is responsive to treatment

with aflibercept. In some embodiments, the subject has been pre-treated (e.g., has received prior treatment) with aflibercept. The volume of a human eye is approximately two times that of the eye of a non-human primate (e.g., African green monkey), the dose (e.g., the number of vector genomes) administered to a first eye of a human is about 2x the dose (e.g., number of vector genomes) administered to a first eye of a non-human primate, and the dose (e.g., the number of vector genomes) administered to a second (e.g., contralateral) eye of a human is about 2x the dose (e.g., number of vector genomes) administered to a second (e.g., contralateral) eye of a non-human primate.

Unit Doses / Therapeutically Effective Doses

[0073] In some embodiments, a unit dose comprises a therapeutically effective amount of vector (e.g., a viral vector). In some embodiments, a therapeutically effective amount of viral vector is an amount sufficient to ensure efficient delivery of the nucleic acid encoding, e.g., an anti-VEGF agent (such as aflibercept), into target cells (such as retinal cells). In some embodiments, a therapeutically effective amount of viral vector is an amount sufficient to ensure that the anti-VEGF agent (such as aflibercept) is delivered to and expressed in the target cells (e.g., retinal cells) at a level that produces a therapeutic effect. Exemplary therapeutic effects of treatment with the anti-VEGF agent (such as aflibercept) include, but are not limited to, the prevention of or delay in the development of one or more symptoms of the ocular disease, alter the course of a symptom disease, slowing the progression of one or more symptoms of the ocular disease, or reversing one or more symptoms of the ocular disease. Methods of monitoring the progression of ocular diseases described elsewhere herein are well known and widely used.

[0074] In some embodiments the unit dose (e.g., therapeutically effective amount) is expressed as the number of vector genomes administered to the subject. In some embodiments, a unit dose (e.g., therapeutically effective amount) of viral vector is between about 1×10^{10} to about 2×10^{10} , between about 2×10^{10} to about 3×10^{10} , between about 3×10^{10} to about 4×10^{10} , between about 4×10^{10} to about 5×10^{10} , between about 5×10^{10} to about 6×10^{10} , between about 6×10^{10} to about 7×10^{10} , between about 7×10^{10} to about 8×10^{10} , between about 8×10^{10} to about 9×10^{10} , between about 9×10^{10} to about 10×10^{10} , between about 1×10^{11} to about 2×10^{11} , between about 2×10^{11} to about 3×10^{11} , between about 3×10^{11} to about 4×10^{11} , between about 4×10^{11} to about 5×10^{11} , between about 5×10^{11} to about 6×10^{11} , between about 6×10^{11} to about 7×10^{11} , between about 7×10^{11} to about 8×10^{11} , between about 8×10^{11} to about 9×10^{11} , between about 9×10^{11} to about 10×10^{11} , between about 1×10^{12} to about 2×10^{12} , between about 2×10^{12} to about 3×10^{12} , between about 3×10^{12} to about 4×10^{12} , between about 4×10^{12} to about 5×10^{12} , between about 5×10^{12} to about 6×10^{12} , between about 6×10^{12} to about 7×10^{12} , between about 7×10^{12} to about 8×10^{12} , between about 8×10^{12} to about 9×10^{12} , between about 9×10^{12} to about 10×10^{12} , between about 1×10^{13} to about 2×10^{13} , between about 2×10^{13} to about 3×10^{13} , between about 3×10^{13} to about 4×10^{13} , between about 4×10^{13} to about 5×10^{13} , between about 5×10^{13} to about 6×10^{13} , between about 6×10^{13} to about 7×10^{13} , between about 7×10^{13} to about 8×10^{13} ,

between about 8×10^{13} to about 9×10^{13} , or between about 9×10^{13} to about 10×10^{13} vector genomes, including any range in between these values.

[0075] In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., an rAAV vector disclosed herein) is about 2.1×10^{12} vector genomes. In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., an rAAV vector disclosed herein) is between about 2×10^{12} to about 6×10^{12} vector genomes. In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., an rAAV vector disclosed herein) is between about 10^{10} to about 10^{13} , between about 10^{10} to about 10^{11} , between about 10^{11} to about 10^{12} , between about 10^{12} to about 10^{13} , between about 10^{13} to about 10^{14} , between about 2×10^{11} to about 4×10^{11} , between about 3×10^{11} to about 5×10^{11} , between about 4×10^{11} to about 6×10^{11} , between about 5×10^{11} to about 7×10^{11} , between about 6×10^{11} to about 8×10^{11} , between about 7×10^{11} to about 9×10^{11} , between about 8×10^{11} to about 10×10^{11} , between about 1×10^{12} to about 3×10^{12} , between about 2×10^{12} to about 4×10^{12} , between about 3×10^{12} to about 5×10^{12} , between about 4×10^{12} to about 6×10^{12} , between about 5×10^{12} to about 7×10^{12} , between about 6×10^{12} to about 8×10^{12} , between about 7×10^{12} to about 9×10^{12} , between about 8×10^{12} to about 10×10^{12} , between about 1×10^{13} to about 5×10^{13} , between about 5×10^{13} to about 1×10^{14} , between about 10^{12} to about 5×10^{12} , or between about 5×10^{12} to about 1×10^{13} vector genomes, including any range in between these values. In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., a rAAV vector disclosed herein such as rAAV2.7m8 or AAV2.5T.LSV) is between about 1×10^{10} to about 1×10^{13} vector genomes. In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., an rAAV vector disclosed herein) is about 1×10^9 to about 1×10^{14} vector genomes.

[0076] In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., an rAAV vector disclosed herein such as rAAV2.7m8) is about 1×10^{10} to about 1×10^{11} vector genomes. In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., a rAAV vector disclosed herein such as rAAV2.7m8) is be about 1×10^8 to about 1×10^{15} vector genomes. In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., a rAAV vector disclosed herein such as rAAV2.7m8) is at least about any one of 1×10^1 , 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} , 1×10^{13} , 1×10^{14} , 1×10^{15} , 1×10^{16} , 1×10^{17} and 1×10^{18} vector genomes, including any range between these values. In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., an rAAV vector disclosed herein such as rAAV2.7m8) is 1×10^8 to 1×10^{15} vector genomes. In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., a rAAV vector disclosed herein such as rAAV2.7m8) is at most about any one of 1×10^1 , 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} , 1×10^{13} , 1×10^{14} , 1×10^{15} , 1×10^{16} , 1×10^{17} and 1×10^{18} vector genomes. In some embodiments, the unit dose (e.g., therapeutically effective

amount) of viral vector (e.g., a rAAV vector disclosed herein such as rAAV2.7m8) is between 10^{10} to 10^{11} , between 10^{11} to 10^{12} , between 10^{10} to 10^{12} , between 10^{12} to 10^{13} , between 10^{11} to 10^{13} , between 10^{12} to 10^{13} , between 10^{12} to 10^{14} , between 10^{11} to 10^{14} , between 10^{11} to 10^{15} , between 10^{12} to 10^{15} , between 10^{13} to 10^{14} , between 10^{14} to 10^{15} , between 10^{15} to 10^{16} , between 10^{16} to 10^{17} , between 10^{17} to 10^{18} , between 10^{18} to 10^{19} , or between 10^{19} to 10^{20} vector genomes, including any range in between these values.

[0077] In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., a rAAV vector disclosed herein such as rAAV2.7m8) is between about 1×10^{10} to 2×10^{10} , between 2×10^{10} to 3×10^{10} , between 3×10^{10} to 4×10^{10} , between 4×10^{10} to 5×10^{10} , between 5×10^{10} to 6×10^{10} , between 6×10^{10} to 7×10^{10} , between 7×10^{10} to 8×10^{10} , between 8×10^{10} to 9×10^{10} , between 9×10^{10} to 10×10^{10} , between 1×10^{11} to 2×10^{11} , between 2×10^{11} to 3×10^{11} , between 2×10^{11} to 2.5×10^{11} , between 2.5×10^{11} to 3×10^{11} , between 3×10^{11} to 4×10^{11} , between 4×10^{11} to 5×10^{11} , between 5×10^{11} to 6×10^{11} , between 6×10^{11} to 7×10^{11} , between 7×10^{11} to 8×10^{11} , between 8×10^{11} to 9×10^{11} , between 9×10^{11} to 10×10^{11} , between 1×10^{12} to 2×10^{12} , between 2×10^{12} to 3×10^{12} , between 2.5×10^{12} to 3×10^{12} , between 3×10^{12} to 4×10^{12} , between 4×10^{12} to 5×10^{12} , between 5×10^{12} to 6×10^{12} , between 6×10^{12} to 7×10^{12} , between 7×10^{12} to 8×10^{12} , between 8×10^{12} to 9×10^{12} , between 9×10^{12} to 10×10^{12} , between 1×10^{13} to 2×10^{13} , between 2×10^{13} to 3×10^{13} , between 3×10^{13} to 4×10^{13} , between 4×10^{13} to 5×10^{13} , between 5×10^{13} to 6×10^{13} , between 6×10^{13} to 7×10^{13} , between 7×10^{13} to 8×10^{13} , between 8×10^{13} to 9×10^{13} , or between 9×10^{13} to 10×10^{13} vector genomes.

[0078] In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., an rAAV vector disclosed herein) is between 2.1×10^{11} or between 2.1×10^{12} vector genomes. In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., an rAAV vector disclosed herein) is between 10^{10} to 10^{13} , between 10^{10} to 10^{11} , between 10^{11} to 10^{12} , between 10^{12} to 10^{13} , or between 10^{13} to 10^{14} vector genomes.

[0079] In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., an rAAV vector disclosed herein) is between 1×10^{10} to 2×10^{10} , between 2×10^{10} to 4×10^{10} , between 3×10^{10} to 5×10^{10} , between 4×10^{10} to 6×10^{10} , between 5×10^{10} to 7×10^{10} , between 6×10^{10} to 8×10^{10} , between 7×10^{10} to 9×10^{10} , between 8×10^{10} to 10^{11} , between 1×10^{11} to 2×10^{11} , between 2×10^{11} to 4×10^{11} , between 3×10^{11} to 5×10^{11} , between 4×10^{11} to 6×10^{11} , between 5×10^{11} to 7×10^{11} , between 6×10^{11} to 8×10^{11} , between 7×10^{11} to 9×10^{11} , between 8×10^{11} to 10×10^{11} , between 1×10^{12} to 3×10^{12} , between 2×10^{12} to 4×10^{12} , between 3×10^{12} to 5×10^{12} , between 4×10^{12} to 6×10^{12} , between 5×10^{12} to 7×10^{12} , between 6×10^{12} to 8×10^{12} , between 7×10^{12} to 9×10^{12} , between 8×10^{12} to 10×10^{12} , between 1×10^{13} to 5×10^{13} , between 5×10^{13} to 10×10^{13} , between 10^{12} to 5×10^{12} , between 5×10^{12} to 1×10^{13} , between 7×10^{12} to 1×10^{13} , between 8×10^{12} to 2×10^{13} , between 9×10^{12} to 2×10^{13} , between 9×10^{12} to 2×10^{13} , between 9×10^{12} to 4×10^{13} , between 1×10^{13} to 3×10^{13} , between 1×10^{13} to 2×10^{13} , between 2×10^{13} to 3×10^{13} , between 3×10^{13} to 4×10^{13} , between 4×10^{13} to 5×10^{13} , between 5×10^{13} to 6×10^{13} ,

between 6×10^{13} to 7×10^{13} , between 7×10^{13} to 8×10^{13} , between 8×10^{13} to 9×10^{13} , or between 8×10^{13} to 1×10^{14} vector genomes.

[0080] In some embodiments, the unit dose (e.g., therapeutically effective amount) of vector genomes is selected from the lower range of values described herein in order to, e.g., avoid aggregation of the viral vector present in the pharmaceutical composition that is administered to the subject. In some embodiments, the unit dose (e.g., therapeutically effective amount) of vector genomes is selected from the higher range of values described herein in order to, e.g., ensure efficient delivery of the therapeutic transgene into target cells. In some embodiments, the unit dose (e.g., therapeutically effective dose) is selected from the higher range of values described herein in order to allow smaller volumes of injection, which can reduce adverse effects associated with intravitreal injection, e.g., elevated intraocular pressure, inflammation, irritation, or pain.

[0081] In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., a rAAV vector disclosed herein such as rAAV2.7m8) is about $1E10$, about $1.5E10$, about $2E10$, about $2.5E10$, about $3E10$, about $3.5E10$, about $4E10$, about $4.5E10$, about $5E10$, about $5.5E10$, about $6E10$, about $6.5E10$, about $7E10$, about $7.5E10$, about $8E10$, about $8.5E10$, about $9E10$, about $9.5E10$, about $1E11$, about $1.5E11$, about $2E11$, about $2.5E11$, about $3E11$, about $3.5E11$, about $4E11$, about $4.5E11$, about $5E11$, about $5.5E11$, about $6E11$, about $6.5E11$, about $7E11$, about $7.5E11$, about $8E11$, about $8.5E11$, about $9E11$, about $9.5E11$, about $1E12$, about $1.3E12$, about $1.5E12$, about $2E12$, about $2.1E12$, about $2.3E12$, about $2.5E12$, about $2.7E12$, about $2.9E12$, about $3E12$, about $3.1E12$, about $3.3E12$, about $3.5E12$, about $3.7E12$, about $3.9E12$, about $4E12$, about $4.1E12$, about $4.3E12$, about $4.5E12$, about $4.7E12$, about $4.9E12$, about $5E12$, about $5.1E12$, about $5.3E12$, about $5.5E12$, about $5.7E12$, about $5.9E12$, about $6E12$, about $6.1E12$, about $6.3E12$, about $6.5E12$, about $6.7E12$, about $6.9E12$, about $7E12$, about $7.1E12$, about $7.3E12$, about $7.5E12$, about $7.7E12$, about $7.9E12$, about $8E12$, about $8.1E12$, about $8.3E12$, about $8.5E12$, about $8.7E12$, about $8.9E12$, about $9E12$, about $9.1E12$, about $9.3E12$, about $9.5E12$, about $9.7E12$, about $9.9E12$, about $1.01E13$, about $1.03E13$, about $1.05E13$, about $1.07E13$, about $1.09E13$, about $1.1E13$, about $1.15E13$, about $1.2E13$, about $1.25E13$, about $1.3E13$, about $1.35E13$, about $1.4E13$, about $1.45E13$, about $1.5E13$, about $1.55E13$, about $1.6E13$, about $1.65E13$, about $1.7E13$, about $1.75E13$, about $1.8E13$, about $1.85E13$, about $1.9E13$, about $1.95E13$, about $2.0E13$, about $2.5E13$, about $3.0E13$, about $3.5E13$, about $4.0E13$, about $4.5E13$, about $5.0E13$, about $5.5E13$, about $6.0E13$, about $6.5E13$, about $7.0E13$, about $7.5E13$, about $8.0E13$, about $8.5E13$, about $9.0E13$, about $9.5E13$, or about $1E14$ vector genomes, including any range in between these values, wherein E is a short-hand for base 10 for exponentiation, and xEy refers to x multiplied by base 10 to the y power/exponent. In some embodiments, the unit dose is (such as comprises) between about $1E9$ to about $3E13$ vector genomes, between about $1E10$ and about $1E13$ vector genomes, between about $1E11$ and $1E13$ vector genomes, between about $1E10$ to about $3E12$ vector genomes, or between about $2E12$ and about $6E12$ vector genomes.

[0082] In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector is expressed as multiplicity of infection (MOI). In some cases, MOI refers to the ratio of vectors or viral genomes to the target cells to which the heterologous nucleic acid (e.g., the nucleic acid encoding the anti-VEGF agent (such as aflibercept)) is delivered. In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., an rAAV vector disclosed herein such as rAAV2.7m8) is an MOI of 1×10^6 . In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., a rAAV vector disclosed herein such as rAAV2.7m8) is an MOI of about any one of 1×10^1 , 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} , 1×10^{13} , 1×10^{14} , 1×10^{15} , 1×10^{16} , 1×10^{17} and 1×10^{18} , including any range in between these values. In some embodiments, the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., an rAAV vector disclosed herein such as rAAV2.7m8) is an MOI between about 1×10^8 and about 1×10^{15} . In some embodiments, a the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., a rAAV vector disclosed herein such as rAAV2.7m8) is an MOI of no more than about any one of 1×10^1 , 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} , 1×10^{11} , 1×10^{12} , 1×10^{13} , 1×10^{14} , 1×10^{15} , 1×10^{16} , 1×10^{17} , and 1×10^{18} , including any range in between these values. In some embodiments, a the unit dose (e.g., therapeutically effective amount) of viral vector (e.g., a rAAV vector disclosed herein such as rAAV2.7m8) is an MOI between 1×10^{10} to 2×10^{10} , between 2×10^{10} to 4×10^{10} , between 3×10^{10} to 5×10^{10} , between 4×10^{10} to 6×10^{10} , between 5×10^{10} to 7×10^{10} , between 6×10^{10} to 8×10^{10} , between 7×10^{10} to 9×10^{10} , between 8×10^{10} to 10^{11} , between 1×10^{11} to 2×10^{11} , between 2×10^{11} to 4×10^{11} , between 3×10^{11} to 5×10^{11} , between 4×10^{11} to 6×10^{11} , between 5×10^{11} to 7×10^{11} , between 6×10^{11} to 8×10^{11} , between 7×10^{11} to 9×10^{11} , between 8×10^{11} to 10×10^{11} , between 1×10^{12} to 3×10^{12} , between 2×10^{12} to 4×10^{12} , between 3×10^{12} to 5×10^{12} , between 4×10^{12} to 6×10^{12} , between 5×10^{12} to 7×10^{12} , between 6×10^{12} to 8×10^{12} , between 7×10^{12} to 9×10^{12} , between 8×10^{12} to 10×10^{12} , between 1×10^{13} to 5×10^{13} , between 5×10^{13} to 10×10^{13} , between 10^{12} to 5×10^{12} , between 5×10^{12} to 1×10^{13} , between 7×10^{12} to 1×10^{13} , between 8×10^{12} to 2×10^{13} , between 9×10^{12} to 2×10^{13} , between 9×10^{12} to 4×10^{13} , between 1×10^{13} to 3×10^{13} , between 1×10^{13} to 2×10^{13} , between 2×10^{13} to 3×10^{13} , between 3×10^{13} to 4×10^{13} , between 4×10^{13} to 5×10^{13} , between 5×10^{13} to 6×10^{13} , between 6×10^{13} to 7×10^{13} , between 7×10^{13} to 8×10^{13} , between 8×10^{13} to 9×10^{13} , or between 8×10^{13} to 1×10^{14} , including any range between these values.

[0083] In some embodiments, a unit dose (e.g., therapeutically effective amount) of viral vector is expressed as pfu (plaque forming units). In some embodiments, a unit dose (e.g., therapeutically effective amount) of viral vector (e.g., an rAAV vector disclosed herein such as rAAV2.7m8) is between about 1×10^8 to about 1×10^{12} pfu. In some embodiments, a unit dose (e.g., therapeutically effective amount) of viral vector (e.g., a rAAV vector disclosed herein such as rAAV2.7m8) is least about any one of 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 , 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , 9×10^9 , 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} , 5×10^{10} , 6×10^{10} ,

7×10^{10} , 8×10^{10} , 9×10^{10} , 1×10^{11} , 2×10^{11} , 3×10^{11} , 4×10^{11} , 5×10^{11} , 6×10^{11} , 7×10^{11} , 8×10^{11} , 9×10^{11} or 1×10^{12} pfu, including any range in between these values. In some embodiments, a unit dose (e.g., therapeutically effective amount) of viral vector (e.g., a rAAV vector disclosed herein such as rAAV2.7m8) is about any one of 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 , 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , 9×10^9 , 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} , 5×10^{10} , 6×10^{10} , 7×10^{10} , 8×10^{10} , 9×10^{10} , 1×10^{11} , 2×10^{11} , 3×10^{11} , 4×10^{11} , 5×10^{11} , 6×10^{11} , 7×10^{11} , 8×10^{11} , 9×10^{11} or 1×10^{12} pfu, including any range in between these values.

[0084] In some embodiments, a therapeutically effective amount of viral vector is the amount sufficient to cause expression of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) in the vitreous fluid to achieve a concentration of at about any one of 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, or 1.0 $\mu\text{g/ml}$, including any range in between these values. In some embodiments, a therapeutically effective amount of viral vector is the amount sufficient to cause expression of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) in the retina to achieve a concentration of at least about 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, or 1.0 $\mu\text{g/ml}$, including any range in between these values. In some embodiments, a therapeutically effective amount of viral vector is the amount sufficient to cause expression of the therapeutic protein (e.g., an anti-VEGF agent such as aflibercept) in the choroid to achieve a concentration of at about any one of 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, or 1.0 $\mu\text{g/ml}$, including any range in between these values.

[0085] In some embodiments, the dose (e.g., unit dose) of viral vector administered to the subject is therapeutically effective if administration of the dose to the subject reduces, stops, or prevents at least one symptom of the ocular disease or disorder. In the cases of ocular diseases or disorders characterized by abnormal (e.g., excessive) angiogenesis, such symptoms include, but are not limited to, e.g., visual distortions (such as impaired color vision, blurred vision, deterioration of central vision) and vision loss. In some embodiments, the dose (e.g., unit dose) of viral vector administered to the subject is therapeutically effective if administration of the dose to the subject results in the maintenance, partial resolution, or complete resolution of one or more clinical features of the disease. For example, the dose (e.g., unit dose) of viral vector administered to the subject is therapeutically effective if administration of the dose to the subject results in (a) complete resolution, partial resolution or maintenance of the ocular disease as measured by optical coherence tomography (OCT); (b) an increase and/or maintenance in best corrected visual acuity (such as assessed by an EDTRS eye chart, Amsler grid, etc.); (c) maintenance or reduction of hyperfluorescence as measured via fluorescein angiography (FA).

Exemplary Vectors Delivering Transgenes to Target Cells

[0086] In some embodiments, delivery of a heterologous nucleic acid encoding an anti-VEGF agent (e.g., aflibercept) to a target cell (e.g., a retinal cell) is performed using any suitable vector (also referred to as “gene delivery” or “gene transfer vehicle”). In some embodiments, the vector, delivery vehicle, gene delivery vehicle, or gene transfer vehicle is a macromolecule or complex of molecules that comprises a heterologous nucleic acid and is capable of delivering the heterologous nucleic acid to a target cell. In some embodiments, the target cell is a retinal cell, e.g., any of the cell types that comprise the retina, such as a photoreceptor, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelium cell). In some embodiments, the target cell is any cell to which the nucleic acid molecule or gene is delivered. In some embodiments, the heterologous nucleic acid encodes an anti-VEGF agent (e.g., aflibercept). In some embodiments, the vector, delivery vehicle, gene delivery vehicle, or gene transfer vehicle is present in a pharmaceutical composition that is formulated or adapted for administration to the eye of a subject (e.g., a human or non-human primate) via intravitreal injection (IVT).

[0087] In some embodiments, the vector is a viral vector, e.g., an adenovirus, an adeno-associated virus (AAV), a retrovirus, or a lentivirus, or a hybrid viral vector. In some embodiments the viral vector is a recombinant viral vector. In some embodiments, the viral vector (e.g., recombinant viral vector) comprises a heterologous nucleic acid (e.g., a heterologous nucleic acid encoding an anti-VEGF agent such as aflibercept) that is operably linked to a strong eukaryotic promoter (e.g., a cytomegalovirus (CMV) promoter or a constitutive promoter). In some embodiments, the viral vector (such as a recombinant viral vector) comprises at least one (such as more than one) nucleic acid molecule. In some embodiments, the at least one (such as more than one) nucleic acid is a DNA (e.g., cDNA) or an RNA. In some embodiments, viral vector (such as a recombinant viral vector) comprises both DNA and RNA. In some embodiments, the RNA is a transcript (e.g., a transcript of an anti-VEGF agent such as aflibercept) that comprises, e.g., introns, untranslated regions (UTRs), termination sequences and the like. In some embodiments, the DNA encodes an anti-VEGF agent (e.g., aflibercept) and optionally further comprises promoter sequences, UTRs, termination sequences, and the like. In some embodiments, the vector is a recombinant adeno-associated virus (rAAV) capable of delivering a heterologous nucleic acid (e.g., a nucleic acid encoding an anti-VEGF agent such as aflibercept) to a target cell (e.g., a retinal cell) in which the heterologous nucleic acid is expressed. In some embodiments, expression of the heterologous nucleic acid exerts therapeutic effect in the target tissue.

[0088] In some embodiments, the vector is a recombinant viral vector derived from adenovirus (Ad) or adeno-associated virus (AAV) that has been altered so that it is replication-defective in the subject (e.g., a human or a non-human primate). In some embodiments the adeno-associated virus (AAV) is a recombinant AAV (rAAV). In some embodiments, the heterologous nucleic acid (e.g., a

nucleic acid that encodes an anti-VEGF agent such as aflibercept) integrates into the target cell genome (e.g., retinal cell genome), resulting in long-term expression of, e.g., the anti-VEGF agent (such as aflibercept), in the target cell. In some embodiments, the viral vector delivers a plasmid or other extrachromosomal genetic element that comprises the heterologous nucleic acid (e.g., a nucleic acid that encodes an anti-VEGF agent such as aflibercept) to the target cell (e.g., retinal cell).

[0089] AAV or rAAV are small non-enveloped single-stranded DNA viruses. rAAVs are non-pathogenic human parvoviruses and can be made to 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, making AAV or rAAV a suitable delivery system for gene therapy. AAV and rAAV used for gene therapy for delivery of an anti-VEGF agent, e.g., aflibercept, can be of any serotype. In some embodiments, pharmaceutical compositions and methods of the disclosure provide for use of any suitable AAV serotype, including AAV1, AAV2, AAV2.5, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, rh10, AAV-DJ, and any hybrid or chimeric AAV thereof. In some embodiments, the serotype used is based on tropism of the virus, or infectivity of a target cell of interest. In some embodiments, AAV2 or rAAV2 is used to deliver a nucleic acid sequence encoding an anti-VEGF agent (e.g., aflibercept) into an eye or retinal cells of a subject via intravitreal or subretinal injection. In some embodiments, rAAV2.7m8 is used to deliver the nucleic acid sequence of the anti-VEGF agent (e.g., aflibercept) into the retinal cells of a subject.

[0090] In some embodiments, AAV or rAAV viruses, particles, or virions comprising a variant capsid protein having increased infectivity of target cells, e.g. retinal cells, are used to increase transduction of retinal cells or to increase targeting of gene delivery to retinal cells in a subject. In some embodiments, the rAAV particle comprises an amino acid modification in a capsid protein GH loop/loop IV of the AAV capsid protein. In some embodiments, the site of modification is a solvent-accessible portion of the GH loop/loop IV of the AAV capsid protein. For a description of the GH loop/loop IV of AAV capsid, see, e.g., van Vliet et al. (2006) *Mol. Ther.* 14:809; Padron et al. (2005) *J. Virol.* 79:5047; and Shen et al. (2007) *Mol. Ther.* 15:1955. Several AAV capsid variants are known, including the 7m8 variant. In some embodiments, a rAAV particle comprises a variant AAV capsid protein that comprises an insertion of from 5 amino acids to 11 amino acids, e.g., 7 amino acid sequence, in the GH loop of a capsid protein relative to a corresponding parental AAV capsid protein, and wherein the variant capsid protein confers increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV particle comprising the corresponding parental or unmodified AAV capsid protein. In some embodiments, any one of the following amino acid sequences can be inserted in the GH loop of a capsid protein: LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8),

LAKDSTDTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), and LASTGKVPNA (SEQ ID NO: 12), LGETTRP (SEQ ID NO: 14), NETITRP (SEQ ID NO: 15), KAGQANN (SEQ ID NO: 16), KDPKTTN (SEQ ID NO: 17), KDTDTR (SEQ ID NO: 18), RAGGSVG (SEQ ID NO: 19), AVDTTKF (SEQ ID NO: 20), and STGKVPN (SEQ ID NO: 21). In some embodiments, any one of the amino acid sequences set forth in SEQ ID NOs: 1-12 and 14-21 is inserted in the solvent-exposed GH loop of VP1 capsid protein in a rAAV. Additional details regarding amino acid sequences that can be inserted into the GH loop of a capsid protein, e.g., to facilitate transduction of a nucleic acid of interest to a retinal cell following IVT injection, are provided in WO2012145601, the contents of which are incorporated herein by reference in their entirety. In some embodiments, rAAV.7m8 comprising aflibercept is used for gene therapy.

[0091] In some embodiments, any one of the following amino acid sequences: LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8), LAKDSTDTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), and LASTGKVPNA (SEQ ID NO: 12), LGETTRP (SEQ ID NO: 14), NETITRP (SEQ ID NO: 15), KAGQANN (SEQ ID NO: 16), KDPKTTN (SEQ ID NO: 17), KDTDTR (SEQ ID NO: 18), RAGGSVG (SEQ ID NO: 19), AVDTTKF (SEQ ID NO: 20), and STGKVPN (SEQ ID NO: 21) can be inserted at the following positions to generate a rAAV variant for use in gene therapy: between positions 587 and 588 of the AAV2 capsid protein; between amino acids 590 and 591 of the AAV1 capsid protein; between amino acids 575 and 576 of the AAV5 capsid protein; between amino acids 590 and 591 of the AAV6 capsid protein; between amino acids 589 and 590 of the AAV7 capsid protein; between amino acids 590 and 591 of the AAV8 capsid protein; between amino acids 588 and 589 of the AAV9 capsid protein; or between amino acids 589 and 590 of the AAV10 capsid protein.

[0092] In some embodiments, AAV or rAAV viruses, particles, or virions comprising a variant capsid protein that are used in gene therapy exhibit one or more of the following characteristics: 1) increased infectivity of a retinal cell; 2) altered tropism; 3) increased binding to heparin and/or heparin sulfate proteoglycans and/or the inner limiting membrane (ILM); and 4) an increased ability to infect and/or deliver a therapeutic gene product across the ILM when administered intravitreally, as compared to a corresponding viral vector comprising its native, wild-type, and/or parental capsid protein. In some embodiments, the variant AAV capsid protein used in gene therapy comprises a modified sequence comprising one or more amino acids substitutions within amino acid residues 570-579 relative to a parental AAV capsid protein, wherein the modified sequence comprises HKFKSGD (SEQ ID NO: 37), and wherein the amino acid residue numbering corresponds to an AAV5 VP1 capsid protein. In some embodiments, the variant AAV capsid protein comprises a modified sequence comprising one or more amino acids substitutions within amino acid residues 570-579 relative to a

parental AAV capsid protein, wherein the modified sequence comprises $X_1X_2HKFKSGDX_3$ (SEQ ID NO:38), and wherein the amino acid residue numbering corresponds to an AAV5 VP1 capsid protein, wherein X_{1-3} can independently be any amino acid. In some embodiments, each of X_{1-3} is independently selected from A, L, G, S, and T. In some embodiments, each of X_{1-3} is independently selected from A, L, G, S, and T. In some embodiments, X_1 is L. In some embodiments, X_2 is A. In some embodiments, X_3 is A. In some embodiments, the variant AAV capsid protein comprises a modified sequence comprising one or more amino acids substitutions within amino acid residues 570-579 relative to a parental AAV capsid protein, wherein the modified sequence comprises LAHKFKSGDA (SEQ ID NO: 39), a sequence having at least 80% or at least 90% homology with SEQ ID NO: 39; having at least 80% or at least 90% sequence identity with SEQ ID NO: 39; or having four or more, five or more, six or more, seven or more, eight or more, or nine or more consecutive amino acids within SEQ ID NO: 39, and wherein the amino acid residue numbering corresponds to an AAV5 VP1 capsid protein. In some embodiments, the modified sequence comprises LAHKFKSGDA (SEQ ID NO: 39).

[0093] In some embodiments, the parental AAV capsid protein is a wild-type AAV capsid protein, for example an AAV type 1 (AAV1), AAV type 2 (AAV2), AAV type 3 (AAV3), AAV type 4 (AAV4), AAV type 5 (AAV5), AAV type 6 (AAV6), AAV type 7 (AAV7), AAV type 8 (AAV8), AAV type 9 (AAV9), AAV type 10 (AAV10), avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, or ovine AAV capsid protein. In some embodiments, the parental AAV capsid protein is an AAV5 capsid protein. In some embodiments, the parental AAV capsid protein is a VP1, VP2, or VP3 capsid protein. In some embodiments, the parental AAV capsid protein is an AAV5 VP1 capsid protein.

[0094] In some embodiments, the parental AAV capsid protein is a variant AAV capsid protein. In some embodiments, the parental AAV capsid protein is a variant of an AAV1, an AAV2, an AAV3, an AAV4, an AAV5, an AAV6, an AAV7, an AAV8, an AAV9, an AAV10, an avian AAV, a bovine AAV, a canine AAV, an equine AAV, a primate AAV, a non-primate AAV, or an ovine AAV capsid protein. In some embodiments, the parental AAV capsid protein is a variant of an AAV5 VP1 capsid protein (SEQ ID NO: 40). In some embodiments, the parental AAV capsid protein is a variant of an AAV5 VP1 capsid protein having at least 90%, at least 95%, at least 98%, at least 99% homology to SEQ ID NO: 40. In some embodiments, the parental AAV capsid protein is a variant of an AAV5 VP1 capsid protein having at least 90%, at least 95%, at least 98%, at least 99% sequence identity to SEQ ID NO: 40. In some embodiments, the parental AAV capsid protein is a hybrid capsid protein. In some embodiments, the parental AAV capsid protein is a hybrid of AAV2 and AAV5. In some embodiments, the parental AAV capsid protein is AAV 2.5T, or a variant thereof having at least 90%, at least 95%, at least 98%, at least 99% homology to an AAV2.5T capsid protein. In some embodiments, the parental AAV capsid protein is AAV 2.5T, or a variant thereof having at

least 90%, at least 95%, at least 98%, at least 99% sequence identity to an AAV2.5T capsid protein. In some embodiments, the parental AAV capsid protein is AAV 2.5T. In some embodiments, the parental AAV capsid protein is a VP1, VP2, or VP3 capsid protein. In some embodiments, the parental AAV capsid protein is an AAV2.5T VP1 capsid protein, or a variant thereof having at least 90%, at least 95%, at least 98%, at least 99% homology to an AAV2.5T VP1 capsid protein (SEQ ID NO: 41). In some embodiments, the parental AAV capsid protein is an AAV2.5T VP1 capsid protein, or a variant thereof having at least 90%, at least 95%, at least 98%, at least 99% sequence identity to an AAV2.5T VP1 capsid protein (SEQ ID NO: 41).

[0095] In some embodiments, the variant AAV capsid protein comprises a capsid sequence having at least 85%, homology to the amino acid sequence set forth in SEQ ID NO: 40 or SEQ ID NO: 41. In some embodiments, the variant AAV capsid protein comprises a capsid sequence having at least 85%, sequence identity to the amino acid sequence set forth in SEQ ID NO: 40 or SEQ ID NO: 41. In some embodiments, the variant AAV capsid protein comprises a capsid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% homology to the amino acid sequence set forth in SEQ ID NO: 42 (AAV5.LSV1 VP1) or SEQ ID NO: 43 (AAV2.5T.LSV1 VP1). In some embodiments, the variant AAV capsid protein comprises a capsid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO: 42 or SEQ ID NO: 43. In some embodiments, the variant AAV capsid protein comprises SEQ ID NO: 42 or SEQ ID NO: 43. In some embodiments, the variant AAV capsid protein comprises SEQ ID NO: 42. In some embodiments, the variant AAV capsid protein comprises SEQ ID NO: 43. The amino acid sequences of SEQ ID NOs: 40, 41, 42, and 43 are provided below:

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MSFVDHPPDW LEEVGEGLRE FLGLEAGPPK PKPNQQHQDQ ARGLVLPGYN YLGPGNGLDR
GEPVNRADDEV AREHDISYNE QLEAGDNPYL KYNHADA EFQ EKLADDT SFG GNLGKAVFQA
KKRVLEPFGL VEEGAKTAPT GKRI DDHFPK RKKARTEEDS KPSTSSDAEA GPSGSQQLQI
PAQPASSLGA DTMSAGGGGP LGDNNQGADG VGNASGDWHC DSTWMGDRVV TKSTRTWVLP
SYNNHQYREI KSGSVDGSNA NAYFGYSTPW GYFDFNRFHS HWSPRDWQRL INNYWGFRPR
SLRVKIFNIQ VKEVTVQDST TTIANNL TST VQVFTDDDYQ LPYVVGNGTE GCLPAFP PQV
FTLPQYGYAT LNRDNTENPT ERS SFFCLEY FPSKMLRTGN NFEFTYNFEE VPFHSSFAPS
QNLFKLANPL VDQYLYRFVS TNNTGGVQFN KNLAGRYANT YKNWFPGPMG RTQGWNLGSG
VNRASVSAFA TTNRMELEGA SYQVPPQPNG MTNNLQGSNT YALENTMIFN SQPANPGTTA
TYLEGNMLIT SESETQPVNR VAYNVGGQMA TNNQSSTTAP ATGTYNLQEI VPGSVWMERD
VYLQGPIWAK IPETGAHFHP SPAMGGFGLK HPPPMMLIKN TPVPGNITSF SDVPVSSFIT
QYSTGQVTVE MEWELKKENS KRWNPEIQYT NNYNDPQFVD FAPDSTGEYR TTRPIGTRYL
TRPL (SEQ ID NO: 40)
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MAADGYLPDW LEDTLSEGIR QWWKLKPGPP PPKPAERHKD DSRGLVLPGY KYLGPFNGLD
KGEPVNEADA AALEHDKAYD RQLDSGDNPY LKYNHADA EF QERLKEDTSF GGNLGRAV FQ
AKKRVLEPFGL LVEEGAKTAP TGKRIDDHFP KRKKARTEED SKPSTSSDAE AGPSGSQQLQ
IPAQPASSLG ADTMSAGGGG PLGDNNQGAD GVGNASGDWH CDSTWMGDRV VTKSTRTWVL
PSYNNHQYRE IKSGSVDGSN ANAYFGYSTP WGYFDFNRFH SHWSPRDWQR LINNYWGFRP
RSLRVKIFNI QVKEVTVQDS TTTIANNLTS TVQVFTDDDY QLPYVVGNGT EGCLPAFP PQ
VFTLPQYGYA TLNRDNTENP TERS SFFCLE YFPSKMLRTG NNFEFTYNFE EVPFHSSFAP
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SQNLFKLANP LVDQYLYRFV STNNTGGVQF NKNLAGRYAN TYKNWFPGPM GRTQGWNLGS
 GVNRAVSFAF ATTNRMELEG ASYQVPPQPN GMTNNLQGSN TYALENTMIF NSQPANPGTT
 ATYLEGNMLI TSESETQPVN RVAYNVGGQM ATNNQSSTTA PTTGTYNLQE IVPGSVWMER
 DVYLQGPPIWA KIPETGAHFH PSPAMGGFGL KHPPPMMLIK NTPVPGNITS FSDVPVSSFI
 TQYSTGQVTV EMEWELKKEN SKRWNPEIQY TNNYNDPQFV DFAPDSTGEY RTTRPIGTRY
 LTRPL (SEQ ID NO: 41)

MSFVDHPPDW LEEVGEGLRE FLGLEAGPPK PKPNQQHQDQ ARGLVLPGYN YLGPGNGLDR
 GEPVNRADDEV AREHDISYNE QLEAGDNPYL KYNHADADEF EKLADDTSTFG GNLGKAVFQA
 KKRVLFPFGL VEEGAKTAPT GKRIDDHFPK RKKARTEEDS KPSTSSDAEA GPSGSQQLQI
 PAQPASSLGA DTMSAGGGGP LGDNNQGADG VGNASGDWHC DSTWMGDRV TKSTRTWVLP
 SYNHNHQRREI KSGSVDGSNA NAYFGYSTPW GYFDFNRFHS HWSPRDWQRL INNYWGFRPR
 SLRVKIFNIQ VKEVTVDST TTIANNLTST VQVFTDDDYQ LPYVVGNGTE GCLPAFPFPQV
 FTLPQYGYAT LNRDNTENPT ERSSFFCLEY FPSKMLRTGN NFEFTYNFEE VPFHSSFAPS
 QNLFKLANPL VDQYLYRFVS TNNTGGVQFN KNLAGRYANT YKNWFPGPMG RTQGWNLGS
 VNRAVSFAFA TTNRMELEGA SYQVPPQPNG MTNNLQGSNT YALENTMIFN SQPANPGTTA
 TYLEGNMLIT SESETQPVNR VAYNVGGQML AHKFKSGDAP ATGTYNLQEI VPGSVWMERD
 VYLQGPPIWAK IPETGAHFHP SPAMGGFGLK HPPPMMLIKN TPVPGNITSF SDVPVSSFIT
 QYSTGQVTV MEWELKKENS KRWNPFIQYT NNYNDPQFVD FAPDSTGEYR TTRPIGTRYL
 TRPL (SEQ ID NO: 42)

MAADGYLPDW LEDTLSEGR QWWKLKPGPP PPKPAERHKD DSRGLVLPGY KYLGPFNGLD
 KGEPVNEADA AALEHDKAYD RQLDSGDNPY LKYNHADADEF QERLKEDTSF GGNLGRAVFO
 AKKRVLFPFG LVEEGAKTAP TGKRIDDHFP KKKARTEED SKPSTSSDAE AGPSGSQQLQ
 IPAQPASSLG ADTMSAGGGG PLGDNNQGAD GVGNASGDWH CDSTWMGDRV VTKSTRTWVL
 PSYNNHQRREI KSGSVDGSN ANAYFGYSTP WGYFDFNRFH SHWSPRDWQR LINNYWGFRP
 RSLRVKIFNI QVKEVTVDST TTTIANNLTS TVQVFTDDDY QLPYVVGNGT EGCLPAFPFPQ
 VFTLPQYGYA TLNRDNTENP TERSFFCLE YFPSKMLRTG NNFEFTYNFE EVPFHSSFAP
 SQNLFKLANP LVDQYLYRFV STNNTGGVQF NKNLAGRYAN TYKNWFPGPM GRTQGWNLGS
 GVNRAVSFAF ATTNRMELEG ASYQVPPQPN GMTNNLQGSN TYALENTMIF NSQPANPGTT
 ATYLEGNMLI TSESETQPVN RVAYNVGGQM LAHKFKSGDA PTTGTYNLQE IVPGSVWMER
 DVYLQGPPIWA KIPETGAHFH PSPAMGGFGL KHPPPMMLIK NTPVPGNITS FSDVPVSSFI
 TQYSTGQVTV EMEWELKKEN SKRWNPEIQY TNNYNDPQFV DFAPDSTGEY RTTRPIGTRY
 LTRPL (SEQ ID NO: 43)

[0096] In some embodiments, the variant AAV capsid protein comprises a capsid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% homology to the amino acid sequence set forth in SEQ ID NO: 44 (AAV5.LSV1 VP2) or SEQ ID NO: 45 (AAV5.LSV1 VP3). In some embodiments, the variant AAV capsid protein comprises a capsid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence set forth in SEQ ID NO: 44 or SEQ ID NO: 45. In some embodiments, the variant AAV capsid protein comprises SEQ ID NO: 44 or SEQ ID NO: 45. In some embodiments, the variant AAV capsid protein comprises SEQ ID NO: 44. In some embodiments, the variant AAV capsid protein comprises SEQ ID NO: 45. The amino acid sequences of SEQ ID NOs: 44 and 45 are provided below:

APTGKRIDDH FPKRKKARTE EDSKPSTSSD AEAGPSGSQQ LQIPAQPASS LGADTMSAGG
 GGPLGDNNQG ADGVGNASGD WHCDSTWMGD RVVTKSTRTW VLPSYNNHQR REIKSGSVDG
 SNANAYFGYS TPWGYFDFNR FHSWSPRDW QRLINNYWGF RPRSLRVKIF NIQVKEVTVD

DSTTTIANNL TSTVQVFTDD DYQLPYVVG N GTEGCLPAFP PQVFTLPQYG YATLNRDNT E
 NPTERSFFC LEYFPSKMLR TGNNFEFTYN FEEVFFHSSF APSQNLFKLA NPLVDQYLYR
 FVSTNNTGGV QFNKNLAGRY ANTYKNWFPG PMGRTQGWN L GSGVNRASVS AFATTNRMEL
 EGASYQVPPQ PNGMTNNLQG SNTYALENTM IFNSQPANPG TTATYLEGNM LITSESETQP
 VNRVAYNVGG QMLAHKFKSG DAPTTGTYNL QEIVPGSVWM ERDVYLQGP I WAKIPETGAH
 FHPSPAMGGF GLKHPPPMML IKNTPVPGNI TSFSDVPVSS FITQYSTGQV TVEMEWELKK
 ENSKRWNPEI QYTNNYNDPQ FVDFAPDSTG EYRTTRPIGT RYLTRPL (SEQ ID NO: 44)

MSAGGGGPLG DNNQGADGVG NASGDWHCDS TWMGDRVVT K STRTWVLPSY NNHQYREIKS
 GSVDGSNANA YFGYSTPWGY FDFNRFHSHW SPRDWQRLIN NYWGFRPRSL RVKIFNIQVK
 EVTVDSTTT IANNLTSTVQ VFTDDDYQLP YVVGNGTEGC LPAFPPQVFT LPQYGYATLN
 RDNTENPTER SSFFCLEYFP SKMLRTGN NF EFTYNFEEVP FHSSFAPSQN LFKLANPLVD
 QYLYRFVSTN NTGGVQFNKN LAGRYANTYK NWFP GPMGRT QGWNLGSGVN RASVS AFATT
 NRMELEGASY QVPPQPNGMT>NNLQGSNTYA LENTMIFNSQ PANPGTTATY LEGNMLITSE
 SETQPVNRVA YNVGGQMLAH KFKSGDAPTT GTYNLQEIVP GSVWMERDVY LQGP I WAKIP
 ETGAHFHPSF AMGGFGLKHP PPMMLIKNTP VPGNITSFSD VPVSSFITQY STGQVTVEME
 WELKKENSKR WNPEIQYTN N YNDPQFVDFA PDSTGEYRTT RPIGTRYLTR PL (SEQ ID
 NO: 45)

[0097] While reference above is made to amino acid modifications of capsid proteins (including specific amino acid substitutions and insertions) using the amino acid numbering corresponding to AAV5 VP1, VP2, or VP3 capsid protein, it is understood that any of these amino acid modifications may also be introduced in the capsid protein of AAVs of other serotypes, *e.g.*, at positions corresponding to those of AAV5 VP1, VP2, or VP3. AAV protein sequences share significant homology and similar amino acid numbering, and the skilled artisan can readily determine amino acid residues in other AAV serotypes that correspond to those specifically described above for AAV5 VP1, VP2, and VP3. Additional details regarding variant AAV capsid proteins that comprise a modified sequence comprising one or more amino acids substitutions within amino acid residues 570-579 (*e.g.*, HKFKSGD (SEQ ID NO: 37)) relative to a parental AAV capsid protein and their uses in gene therapy are detailed in USSN 62/839,548 and USSN 62/923,924, the contents of which are incorporated by reference herein in their entirety.

[0098] In some embodiments, the heterologous nucleic acid that encodes an anti-VEGF agent (*e.g.*, aflibercept) is under the transcriptional control of a promoter that initiates transcription of the heterologous nucleic acid. In some embodiments, the promoter is a “strong” or constitutively active promoter, *e.g.*, a cytomegalovirus (CMV) promoter, an elongation factor 1 alpha (EF1a) promoter, a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) promoter, or a connexin36 (or “Cx36”) promoter. In some embodiments, the promoter is a tissue-specific promoter that is activated in specific tissues or cells, such as retinal cells, to reduce potential toxicity or undesirable effects to non-targeted cells. In some embodiments, a recombinant virus and/or plasmid used to generate a rAAV virus comprises other transcriptional or regulatory elements, such as a poly A (polyadenylation) sequence, untranslated regions (UTRs), 3’ UTRs, or termination sequences. In some embodiments, more than one gene is expressed from the vector or plasmid using internal ribosome entry site (IRES)

or similar element that allows co-expression of two or more proteins or create multigene, or polycistronic mRNA.

[0099] In some embodiments, the rAAV and/or plasmid used to generate the rAAV comprises one or more of the following nucleic acid elements: a first ITR sequence; a promoter sequence; an intron sequence; a first UTR sequence; a heterologous nucleic acid encoding an anti-VEGF agent (e.g., aflibercept); a second UTR sequence; a polyA sequence; and a second ITR sequence. In some embodiments, linker sequence(s) are inserted between two or more of the nucleic acid elements. In some embodiments, the heterologous nucleic acid encoding a therapeutic polypeptide encodes aflibercept (or a functional fragment or functional variant thereof).

[0100] In some embodiments, a self-complementary vector (sc) can 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.

[0101] In some aspects, several AAV vectors may be generated to allow selection of the most optimal serotype and promoter for use with the anti-VEGF agent transgene (e.g., aflibercept transgene).

[0102] In some embodiments, the AAV vector comprises a polynucleotide cassette for enhanced expression of a transgene (e.g., an anti-VEGF agent such as aflibercept) in a target cell (e.g., a retinal cell). In some embodiments, the polynucleotide cassette comprises in 5' to 3' order: (a) a first enhancer region comprising a CMV sequence (SEQ ID NO: 22); (b) a promoter region, comprising a CMV sequence (SEQ ID NO: 23); (c) a 5'UTR region comprising, in 5' to 3' order, TPL and eMLP sequences (SEQ ID NO: 24 and SEQ ID NO: 25, respectively); (d) a coding sequence encoding a peptide or polypeptide (e.g., an anti-VEGF agent such as aflibercept); (e) a second enhancer region comprising a full EES sequence (SEQ ID NO: 26); and (f) a HGH polyadenylation site (SEQ ID NO: 27). In certain of these embodiments, the polynucleotide cassette comprises one or more sequences selected from SEQ ID NO: 28-32 or a sequence with at least 85% identity thereto. In certain of these embodiments the 5' arm of the polynucleotide cassette comprises or consists of SEQ ID NO: 33 or a sequence with at least 85% identity thereto. In certain of these embodiments the 3' arm of the polynucleotide cassette comprises or consists of SEQ ID NO: 34 or a sequence with at least 85% identity thereto. The nucleic acid sequences of SEQ ID NOs: 22-34 are provided below:

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ACTTACGGTA AATGGCCCCG CTGGCTGACC GCCCAACGAC CCCC GCCCAT TGACGTCAAT
AATGACGTAT GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC AATGGGTGGA
GTATTTACGG TAAACTGCCC ACTTGCCAGT ACATCAAGTG TATCATATGC CAAGTCCGCC
CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT TATGCCAGT ACATGACCTT
ACGGGACTTT CCTACTGGC AGTACATCTA CGTATTAGTC ATCGCTATTA CCA (SEQ ID
NO: 22)

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TGCTGATGCG GTTTTGGCAG TACACCAATG GCGGTGGATA GCGGTTTGAC TCACGGGGAT
 TTCCAAGTCT CCACCCCAT T GACGTCAATG GGAGTTTGTT TTGGCACCAA AATCAACGGG
 ACTTTCCAAA ATGTCGTAAT AACCCCGCCC CGTTGACGCA AATGGGCGGT AGGCGGTGAC
 GGTGGGAGGT CTATATAAGC AGAGCTCGTT TAGTGAACCG (SEQ ID NO: 23)

CTCACTCTCT TCCGCATCGC TGTCTGCGAG GGCCAGCTGT TGGGCTCGCG GTTGAGGACA
 AACTCTTCGC GGTCTTTCCA GTACTCTTGG ATCGGAAACC CGTCGGCCTC CGAACGGTAC
 TCCGCCACCG AGGGACCTGA GCGAGTCCGC ATCGACCGGA TCGGAAAACC TCTCGAGAAA
 GCGGTCTAAC CAGTCACAGT CGCAAGGTAG GCTGAGCACC GTGGCGGGCG GCAGCGGGTG
 GCGGTGCGGG TTGTTTCTGG CGGAGGTGCT GCTGATGATG TAATTAAAGT AGGCGGTCTT
 GAGACGGCGG ATGGTCGA (SEQ ID NO: 24)

CCAGCTGTTG GGGTGAGTAC TCCCTCTCAA AAGCGGGCAT TACTTCTGCG CTAAGATTGT
 CAGTTTCCAA AAACGAGGAG GATTTGATAT TCACCTGGCC CG (SEQ ID NO: 25)

CTGTTCTCAT CACATCATAT CAAGGTTATA TACCATCAAT ATTGCCACAG ATGTTACTTA
 GCCTTTTAAAT ATTTCTCTAA TTTAGTGTAT ATGCAATGAT AGTTCTCTGA TTTCTGAGAT
 TGAGTTTCTC ATGTGTAATG ATTATTTAGA GTTTCTCTTT CATCTGTTCA AATTTTTGTC
 TAGTTTATTT TTTTACTGAT TTGTAAGACT TCTTTTTATA ATCTGCATAT TACAATTCTC
 TTTACTGGGG TGTGCAAAT ATTTTCTGTC ATTCTATGGC CTGACTTTTC TTAATGGTTT
 TTTAATTTTA AAAATAAGTC TTAATATTCA TGCAATCTAA TTAACAATCT TTTCTTTGTG
 GTTAGGACTT TGAGTCATAA GAAATTTTTT TCTACACTGA AGTCATGATG GCATCACTTCT
 ATATTATTTT CTAAAAGATT TAAAGTTTTG CCTTCTCCAT TTAGACTTAT AATTCAGTGG
 AATTTTTTTT TGTGTATGGT ATGACATATG GGTTCCCTTT TATTTTTTAC ATATAAATAT
 ATTTCCCTGT TTTTCTAAAA AAGAAAAAGA TCATCATTTT CCCATTGTAA AATGCCATAT
 TTTTTTCATA GGTCACCTTAC ATATATCAAT GGGTCTGTTT CTGAGCTCTA CTCTATTTTA
 TCAGCCTCAC TGTCTATCCC CACACATCTC ATGCTTTGCT CTAAATCTTG ATATTTAGTG
 GAACATTCTT TCCCATTTTG TTCTACAAGA ATATTTTTGT TATTGTCTTT GGGCTTTCTA
 TATACATTTT GAAATGAGGT TGACAAGTTA (SEQ ID NO: 26)

CTGCCCCGGT GGCATCCCTG TGACCCCTCC CCAGTGCCTC TCCTGGCCCT GGAAGTTGCC
 ACTCCAGTGC CCACCAGCCT TGTCCATAA AAATTAAGTT GCATCATTTT GTCTGACTAG
 GTGTCCCTTCT ATAATATTAT GGGGTGGAGG GGGGTGGTAT GGAGCAAGGG GCCCAAGTTG
 GGAAGAAACC TGTAGGCCCT GC (SEQ ID NO: 27)

AGGCGGTCTT GAGACGGCGG ATGGTCGAGG TGAGGTGTGG CAGGCTTGAG ATCCAGCTGT
 TGGGGTGA (SEQ ID NO: 28)

CGCTGTTTTG ACCTCCATAG TGGACACCGG GACCGATCCA GCCTCCGCGT CTCAGGGGAG
 ATCTCGTTTA GTGAACCGTC AGATCCTCAC TCTCTTCCGC ATCGCTGTCT GCGAGGGCCA
 GCTGTTGGG (SEQ ID NO: 29)

TTGATATTCA CCTGGCCCGA TCTGGCCATA CACTTG (SEQ ID NO: 30)

CCCAGGTCCA AGTTTAAACG CC (SEQ ID NO: 31)

TCTTTGGGCT TTCTATATAC ATTTTGAAAT GAGGTTGACA AGTTACCTAG GAAAACGTGTC
 TTCTGCCCCG GGTGGCA (SEQ ID NO: 32)

CTCTGGAGAC GACTTACGGT AAATGGCCCG CCTGGCTGAC CGCCCAACGA CCCCCGCCCA
 TTGACGTCAA TAATGACGTA TGTTCCCATATA GTAACGCCAA TAGGGACTTT CCATTGACGT
 CAATGGGTGG AGTATTTACG GTAAACTGCC CACTTGGCAG TACATCAAGT GTATCATATG
 CCAAGTCCGC CCCCTATTGA CGTCAATGAC GGTAATGGC CCGCCTGGCA TTATGCCAG
 TACATGACCT TACGGGACTT TCCTACTTGG CAGTACATCT ACGTATTAGT CATCGCTATT
 ACCATGCTGA TGCGGTTTTG GCAGTACACC AATGGGCGTG GATAGCGGTT TGACTCACGG

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GGATTTCCAA GTCTCCACCC CATTGACGTC AATGGGAGTT TGTTTTGGCA CAAAATCAA
CGGGACTTTC CAAAATGTCG TAATAACCCC GCCCCGTTGA CGCAAATGGG CGGTAGGCGT
GTACGGTGGG AGGTCTATAT AAGCAGAGCT CGTTTAGTGA ACCGTCAGAT CGCCTGGAGA
GGCCATCCAC GCTGTTTTGA CCTCCATAGT GGACACCGGG ACCGATCCAG CCTCCGCGTC
TCAGGGGAGA TCTCGTTTAG TGAACCGTCA GATCCTCACT CTCTTCCGCA TCGCTGTCTG
CGAGGGCCAG CTGTTGGGCT CGCGGTTGAG GACAAACTCT TCGCGGTCTT TCCAGTACTC
TTGGATCGGA AACCCGTCGG CCTCCGAACG GTACTCCGCC ACCGAGGGAC CTGAGCGAGT
CCGCATCGAC CGGATCGGAA AACCTCTCGA GAAAGGCGTC TAACCAGTCA CAGTCGCAAG
GTAGGCTGAG CACCGTGGCG GCGGCGAGCG GGTGGCGGTC GGGGTTGTTT CTGGCGGAGG
TGCTGCTGAT GATGTAATTA AAGTAGGCGG TCTTGAGACG GCGGATGGTC GAGGTGAGGT
GTGGCAGGCT TGAGATCCAG CTGTTGGGGT GAGTACTCCC TCTCAAAGC GGGCATTACT
TCTGCGCTAA GATTGTCAGT TTCCAAAAAC GAGGAGGATT TGATATTAC CTGGCCCGAT
CTGGCCATAC ACTTGAGTGA CAATGACATC CACTTTGCCT TTCTCTCCAC AGGTGTCCAC
TCCCAGGTCC AAGTTTAAAC GCCGCCACCA TG (SEQ ID NO: 33)

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ACTGTTCTCA TCACATCATA TCAAGGTTAT ATACCATCAA TATTGCCACA GATGTTACTT
AGCCTTTTAA TATTTCTCTA ATTTAGTGTG TAGTCAATGA TAGTTCTCTG ATTTCTGAGA
TTGAGTTTCT CATGTGTAAT GATTATTTAG AGTTTCTCTT TCATCTGTTC AAATTTTTGT
CTAGTTTTAT TTTTACTGA TTTGTAAGAC TTCTTTTAT AATCTGCATA TTACAATTCT
CTTTACTGGG GTGTTGCAAA TATTTTCTGT CATTCTATGG CCTGACTTTT CTTAATGGTT
TTTTAATTTT AAAAATAAGT CTTAATATTC ATGCAATCTA ATTAACAATC TTTTCTTTGT
GGTTAGGACT TTGAGTCATA AGAAATTTTT CTCTACACTG AAGTCATGAT GGCATGCTTC
TATATTATTT TCTAAAAGAT TTAAAGTTTT GCCTTCTCCA TTTAGACTTA TAATTCACCTG
GAATTTTTTT GTGTGTATGG TATGACATAT GGGTTCCTT TTATTTTTTA CATATAAATA
TATTTCCCTG TTTTCTAAA AAAGAAAAAG ATCATCATTT TCCCATTGTA AAATGCCATA
TTTTTTTCAT AGGTCACCTA CATATATCAA TGGGTCTGTT TCTGAGCTCT ACTCTATTTT
ATCAGCCTCA CTGTCTATCC CCACACATCT CATGCTTTGC TCTAAATCTT GATATTTAGT
GGAACATTCT TTCCCATTTT GTTCTACAAG AATATTTTTG TTATTGTCTT TGGGCTTTCT
ATATACATTT TGAAATGAGG TTGACAAGTT ACCTAGGAAA ACTGTCTTCC TGCCCGGGTG
GCATCCCTGT GACCCCTCCC CAGTGCCTCT CCTGGCCCTG GAAGTTGCCA CTCCAGTGCC
CACCAGCCTT GTCCTAATAA AATTAAGTTG CATCATTTTG TCTGACTAGG TGTCCTTCTA
TAATATTATG GGGTGGAGGG GGGTGGTATG GAGCAAGGGG CCAAGTTGG GAAGAAACCT
GTAGGGCCTG CGAAGACAGT CAG (SEQ ID NO: 34)

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[0103] Additional polynucleotide cassettes for enhanced expression of a transgene (e.g., a transgene encoding an anti-VEGF agent such as aflibercept) in a target cell (such as a retinal cell) are disclosed in WO 2018/170473, the contents of which are incorporated herein by reference in their entirety.

[0104] In some embodiments, the vector is a targeted vector, especially a targeted rAAV (e.g., AAV2.7m8) that shows higher infectivity of a specific cell, such as a retinal cell (e.g., a photoreceptor, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelium cell). Viral vectors for use in the disclosure can include those that exhibit low toxicity and/or low immunogenicity in a subject and expresses therapeutically effective quantities of the anti-VEGF agent (e.g., aflibercept) in a subject, e.g., human patient. Any suitable method known in the art can be used in the biochemical purification of recombinant viruses (e.g., rAAV), e.g., for the preparation of pharmaceutical compositions described elsewhere herein. Recombinant AAV viruses can be harvested directly from cells, or from the culture media comprising cells. Virus can be purified using various biochemical means, such as gel filtration, filtration,

chromatography, affinity purification, gradient ultracentrifugation, or size exclusion methods. In some embodiments, the virus is lyophilized.

[0105] In some embodiments, the rAAV comprises a 7m8 variant capsid protein, or rAAV2.7m8, and a nucleic acid sequence that encodes an anti-VEGF agent (e.g., aflibercept, or a functional fragment or functional variant thereof) in a subject (e.g., human or a non-human primate).

[0106] In some embodiments, the increase in retinal cell infectivity of rAAV variant (e.g., the 7m8 variant) is at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein. In some embodiments, the increase in infectivity of retinal cells is an increase of between 5% to 100%, between 5% to 95%, between 5% to 90%, between 5% to 85%, between 5% to 80%, between 5% to 75%, between 5% to 70%, between 5% to 65%, between 5% to 60%, between 5% to 55%, between 5% to 50%, between 5% to 45%, between 5% to 40%, between 5% to 35%, between 5% to 30%, between 5% to 25%, between 5% to 20%, between 5% to 15%, between 5% to 10% as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0107] In some embodiments, the increase in retinal cell infectivity of a rAAV variant is at least 1-fold, at least 1.1-fold, at least 1.2-fold, at least 1.3-fold, at least 1.4-fold, at least 1.5-fold, at least 1.6-fold, at least 1.7-fold, at least 1.8-fold, at least 1.9-fold, or at least 2-fold compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein. In some embodiments, the increase in infectivity is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold as compared to an AAV particle comprising the corresponding parental AAV capsid protein. In some embodiments, the increase in infectivity is at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 35-fold, at least 40-fold, at least 45-fold, at least 50-fold, at least 55-fold, at least 60-fold, at least 65-fold, at least 70-fold, at least 75-fold, at least 80-fold, at least 85-fold, at least 90-fold, or at least 100-fold compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0108] In some embodiments, the increase in retinal cell infectivity is between 10-fold to 100-fold, between 10-fold to 95-fold, between 10-fold to 90-fold, between 10-fold to 85-fold, between 10-fold to 80-fold, between 10-fold to 75-fold, between 10-fold to 70-fold, between 10-fold to 65-fold, between 10-fold to 60-fold, between 10-fold to 55-fold, between 10-fold to 50-fold, between 10-fold to 45-fold, between 10-fold to 40-fold, between 10-fold to 35-fold, between 10-fold to 30-fold, between 10-fold to 25-fold, between 10-fold to 20-fold, or between 10-fold to 15-fold as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0109] In some embodiments, the increase in retinal cell infectivity is between 2-fold to 20-fold, between 2-fold to 19-fold, between 2-fold to 18-fold, between 2-fold to 17-fold, between 2-fold to 16-fold, between 2-fold to 15-fold, between 2-fold to 14-fold, between 2-fold to 13-fold, between 2-fold to 12-fold, between 2-fold to 11-fold, between 2-fold to 10-fold, between 2-fold to 9-fold, between 2-fold to 8-fold, between 2-fold to 7-fold, between 2-fold to 6-fold, between 2-fold to 5-fold, between 2-fold to 4-fold, or between 2-fold to 3-fold as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0110] In some embodiments, an amino acid modification of a capsid protein described herein can confer an increase in an ability to cross an internal limiting membrane (ILM) in an eye of a primate or human subject as compared to the ability of an AAV particle comprising the corresponding parental or unmodified AAV capsid protein to cross the ILM in the eye of the subject. In some embodiments, the increase in the ability to cross the ILM is an increase of at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein. In some embodiments, the increase in the ability to cross the ILM is an increase of between 5% to 100%, between 5% to 95%, between 5% to 90%, between 5% to 85%, between 5% to 80%, between 5% to 75%, between 5% to 70%, between 5% to 65%, between 5% to 60%, between 5% to 55%, between 5% to 50%, between 5% to 45%, between 5% to 40%, between 5% to 35%, between 5% to 30%, between 5% to 25%, between 5% to 20%, between 5% to 15%, or between 5% to 10% as compared to the parental or unmodified AAV capsid protein.

[0111] In some embodiments, the increase in the ability to cross the ILM is at least 1-fold, at least 1.1-fold, at least 1.2-fold, at least 1.3-fold, at least 1.4-fold, at least 1.5-fold, at least 1.6-fold, at least 1.7-fold, at least 1.8-fold, at least 1.9-fold, or at least 2-fold compared to an AAV particle comprising the corresponding parental AAV capsid protein. In some embodiments, the increase in the ability to cross the ILM is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold as compared to an AAV particle comprising the corresponding parental AAV capsid protein. In some embodiments, the increase in the ability to cross the ILM is at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 35-fold, at least 40-fold, at least 45-fold, at least 50-fold, at least 55-fold, at least 60-fold, at least 65-fold, at least 70-fold, at least 75-fold, at least 80-fold, at least 85-fold, at least 90-fold, or at least 100-fold compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0112] In some embodiments, the increase in the ability to cross the ILM is between 10-fold to 100-fold, between 10-fold to 95-fold, between 10-fold to 90-fold, between 10-fold to 85-fold, between 10-fold to 80-fold, between 10-fold to 75-fold, between 10-fold to 70-fold, between 10-fold to 65-fold, between 10-fold to 60-fold, between 10-fold to 55-fold, between 10-fold to 50-fold, between 10-

fold to 45-fold, between 10-fold to 40-fold, between 10-fold to 35-fold, between 10-fold to 30-fold, between 10-fold to 25-fold, between 10-fold to 20-fold, or between 10-fold to 15-fold as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

[0113] In some embodiments, the increase in the ability to cross the ILM is between 2-fold to 20-fold, between 2-fold to 19-fold, between 2-fold to 18-fold, between 2-fold to 17-fold, between 2-fold to 16-fold, between 2-fold to 15-fold, between 2-fold to 14-fold, between 2-fold to 13-fold, between 2-fold to 12-fold, between 2-fold to 11-fold, between 2-fold to 10-fold, between 2-fold to 9-fold, between 2-fold to 8-fold, between 2-fold to 7-fold, between 2-fold to 6-fold, between 2-fold to 5-fold, between 2-fold to 4-fold, or between 2-fold to 3-fold as compared to an AAV particle comprising the corresponding parental or unmodified AAV capsid protein.

Exemplary Anti-VEGF Agents for Delivery to Target Cells

[0114] In some embodiments, a gene therapy is used to deliver a transgene comprising a nucleic acid sequence that encodes or expresses an anti-VEGF agent when administered via intravitreal (IVT) injection to a subject (e.g., a human or a non-human primate). In some embodiments, rAAV comprising a capsid variant (e.g., AAV2.7m8) described herein comprises a heterologous nucleic acid sequence that encodes an anti-VEGF agent is used to deliver the sequence of the anti-VEGF agent gene into retinal cells upon intravitreal to a subject. In some embodiments, the rAAV comprising the gene encoding the anti-VEGF agent is formulated for gene therapy and intravitreal injection. In some embodiments, the gene encoding the anti-VEGF agent refers to a functional fragment or a functional variant thereof. In some embodiments, a “functional fragment” and/or a “functional variant” of an anti-VEGF agent refers to a fragment or variant of an anti-VEGF agent that is capable of producing a therapeutic effect when administered to the subject.

[0115] In some embodiments, the anti-VEGF agent is any therapeutic agent, including proteins, polypeptides, peptides, fusion protein, multimeric proteins, gene products, antibody, human monoclonal antibody, antibody fragment, aptamer, kinase inhibitor, receptor or receptor fragment, or nucleic acid molecule, that can reduce, interfere with, disrupt, block and/or inhibit the activity or function of an endogenous VEGF and/or an endogenous VEGF receptor (VEGFR), or the VEGF-VEGFR interaction or pathway in vivo. In some embodiments, the anti-VEGF agent is any one of the known therapeutic agents that can reduce new blood vessel growth or formation and/or edema, or swelling, when delivered into a cell, tissue, or a subject in vivo, e.g., ranibizumab, brolucizumab, or bevacizumab. In some embodiments, the anti-VEGF agent is naturally occurring, non-naturally occurring, or synthetic. In some embodiments, the anti-VEGF agent can be derived from a naturally occurring molecule that was subsequently modified or mutated to confer an anti-VEGF activity. In some embodiments, the anti-VEGF agent is a fusion or chimeric protein. In such proteins, functional domains or polypeptides are artificially fused to a moiety or a polypeptide to make a fusion or

chimeric protein that can sequester VEGF in vivo or function as a VEGFR decoy. In some embodiments, the anti-VEGF agent is a fusion or chimeric protein that blocks endogenous VEGFR from interacting with its ligands.

[0116] In some embodiments, the anti-VEGF agent is bevacizumab. Bevacizumab (CAS Registry No. 216974-75-3; Drugbank Accession No. DB00112) is a recombinant humanized monoclonal IgG1 antibody that binds to all VEGF-A isoforms and blocks angiogenesis by inhibiting VEGF-A. Los, M.; Roodhart, J.M. L.; Voest, E. E. (2007). "Target Practice: Lessons from Phase III Trials with Bevacizumab and Vatalanib in the Treatment of Advanced Colorectal Cancer". The Oncologist. 12 (4): 443-50; Shih, T; Lindley, C (November 2006). "Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies." Clinical therapeutics. 28 (11): 1779-802.

[0117] In some embodiments, the anti-VEGF agent is ranibizumab. Ranibizumab (CAS Registry No. 347396-82-1; DrugBank Accession No. DB01270) is a recombinant humanized IgG 1 kappa isotype monoclonal antibody fragment (Fab) and binds to all VEGF-A isoforms with a higher affinity than bevacizumab.

[0118] In some embodiments, the anti-VEGF agent is brotucizumab. Brotucizumab (CAS Registry No. 1531589-13-5) is a humanized single-chain antibody fragment (scFv) that binds all VEGF-A isoforms with high affinity.

[0119] In some embodiments, the anti-VEGF agent is aflibercept. The amino acid sequence of aflibercept is known in the art: C₄₃₁₈H₆₇₈₈N₁₁₆₄O₁₃₀₄S₃₂, FDA Unique Ingredient Identifier (UNII) is 15C2VL427D. The amino acid sequence of aflibercept is available at DrugBank, accession number DB08885:

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SDTGRPFVEM YSEIPEIIHM TEGRELVIPC RVTSPNITVT LKKFPLDTLI PDGKRIIWDS
RKGFIISNAT YKEIGLLTCE ATVNGHLYKT NYLTHRQTNT IIDVVLSPSH GIELSVGEKL
VLNCTARTEL NVGIDFNWEY PSSKHQHKKL VNRDLKTQSG SEMKKFLSTL TIDGVTRSDQ
GLYTCAASSG LMTKKNSTFV RVHEKDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR
TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN
GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS
DIAVEWESNG QPENNYKTP PVLDSGGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNNH
YTQKSLSLSP G (SEQ ID NO: 35)
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[0120] The nucleic acid sequence of aflibercept (SEQ ID NO: 36) is provided in **FIG. 6**.

[0121] As used herein, "aflibercept" refers to a polypeptide or protein sequence, or a functional fragment or variant or mutant thereof, with at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more, or 100% homology to the aflibercept amino acid sequence identified above. Homology refers to the % conservation of residues of an alignment between two sequences, including, but not limited to

functional fragments, sequences comprising insertions, deletions, substitutions, pseudofragments, pseudogenes, splice variants or artificially optimized sequences.

[0122] In some embodiments, the amino acid sequence of aflibercept is at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% homologous to the aflibercept amino acid sequence of SEQ ID NO: 35. In some embodiments, the nucleic acid sequence used in a gene therapy or rAAV disclosed herein is compared to the corresponding cDNA sequence of the aflibercept amino acid sequence identified above, and shows at least 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% sequence homology between the nucleic acid sequences of aflibercept (e.g., SEQ ID NO: 36). In some embodiments, aflibercept is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% spatially homologous to aflibercept (e.g., in terms of its secondary, tertiary, and quaternary structure or conformation). In some embodiments, aflibercept of the pharmaceutical compositions and methods disclosed herein is at most 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% spatially homologous to the aflibercept used in the standard of care (e.g., secondary, tertiary, and quaternary structure or conformation).

[0123] In some embodiments, the aflibercept gene product, or aflibercept transgene, as included in a gene therapy based on a rAAV comprises a capsid variant as disclosed herein (e.g., the 7m8 variant), encodes a protein, fusion protein, or polypeptide that has at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 100% homology to the above amino acid sequence of SEQ ID NO: 35, or between the corresponding cDNA sequences of aflibercept (e.g., cDNA of aflibercept sequence used in a gene therapy compared to SEQ ID NO: 36). In some embodiments, methods and pharmaceutical compositions disclosed herein comprise a functional fragment of aflibercept, or a variant or mutant thereof. In some embodiments, the nucleic acid sequence of aflibercept is modified or codon-optimized to enhance its activity, expression, stability, and/or solubility in vivo.

[0124] In some embodiments, the nucleic acid sequence of aflibercept is derived from its amino acid sequence. In some embodiments, the nucleic acid sequence of aflibercept is codon optimized to improve its expression in a subject.

[0125] Codon optimization can be achieved with any method known in the art. Codon optimization refers to a process of modifying a nucleic acid sequence for enhanced expression of a gene in target or host cells of interest, e.g., human retinal cells, by replacing at least one codon (e.g., about or more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, 100 or more codons) of a native sequence with

codons that are used more frequently or are most frequently used in the host cell while maintaining the native amino acid sequence. Codon usage tables are readily available, including for examples, GenScript Codon Usage Frequency Table Tool at [www\(dot\)genscript\(dot\)com/tools/codon-frequency-table](http://www(dot)genscript(dot)com/tools/codon-frequency-table); Codon Usage Database at [www\(dot\)kazusa\(dot\)or\(dot\)jp/codon/](http://www(dot)kazusa(dot)or(dot)jp/codon/); and Nakamura, Y., et al. "Codon usage tabulated from the international DNA sequence databases: status for the year 2000" Nucl. Acids Res. 28:292 (2000).

[0126] Aflibercept is a 115 kDa fusion protein, which can be glycosylated. Aflibercept comprises an IgG backbone fused to extracellular VEGF receptor sequences of the human VEGFR-1 and VEGFR-2, and functions like a soluble decoy receptor by binding VEGF-A with a greater affinity than its natural or endogenous receptors. See, for example, Stewart MW. Aflibercept (VEGF Trap-eye): the newest anti-VEGF drug. Br. J. Ophthalmol. 2012 Sep;96(9):1157-8. Aflibercept's high affinity for VEGF interferes or disrupts subsequent binding and activation of native or endogenous VEGF receptors. Reduced VEGF activity can lead to decreased angiogenesis and vascular permeability. Inhibition of placental growth factor PlGF and VEGF-B by aflibercept may also contribute to the treatment of ocular diseases or disorders characterized by abnormal (e.g., excessive) angiogenesis and/or neovascularization. PlGF has been associated with angiogenesis and certain ocular diseases or disorders, such as wet AMD, may be associated with elevated levels of PlGF. VEGF-B overexpression can be associated with breakdown of the blood-retinal barrier and retinal angiogenesis. Thus, inhibition of VEGF-A, VEGF-B, and PlGF may all contribute to the efficacy of aflibercept.

[0127] In some embodiments, the nucleic acid sequence of aflibercept is codon-optimized for expression in a primate or a human subject. Construction of a synthetic gene corresponding to the aflibercept amino acid sequence has been described in literature, e.g., Kanda A, Noda K, Saito W, Ishida S. Aflibercept Traps Galectin-1, an Angiogenic Factor Associated with Diabetic Retinopathy. Scientific Reports 5:17946 (2015) (describing "VEGF-Trap_{R1R2} (corresponding to aflibercept) cDNA was generated as a synthetic gene by IDT (Coralville, IA)"). Given the available amino acid sequence of aflibercept, any method known in the art can be used to generate the cDNA of aflibercept for use in a gene therapy or a rAAV described herein.

[0128] In some embodiments, AAV2.7m8 is used as a gene therapy or delivery system for aflibercept. AAV2.7m8-aflibercept refers to a rAAV2 comprising the 7m8 insertion between positions 587 and 588 in capsid protein VP1 of rAAV2 and a nucleic acid sequence encoding aflibercept.

Ocular Diseases or Disorders

[0129] In some embodiments, the methods provided herein are suitable for use in the treatment of ocular diseases or disorders that arise asynchronously in each eye. Examples of diseases that may arise asynchronously (arising first in one eye, and later or not at all in the second eye) include

glaucoma, wAMD, RVO, DME, DR and others. In some embodiments, the methods provided herein are suitable for use in the treatment of ocular diseases or disorders where simultaneous treatment of both eyes is unfeasible, inadvisable, and/or unsafe. In some embodiments, an rAAV particle of any serotype comprising the 7m8 variant (e.g., rAAV2.7m8) or a pharmaceutical composition thereof as described herein is used to treat or at least partially ameliorate an ocular disease or disorder associated with abnormal (e.g., excessive) neovascularization of the eye, e.g., wherein abnormal (e.g., excessive) neovascularization arises asynchronously in each eye. In some embodiments, a rAAV particle comprising a capsid variant protein is used to deliver an anti-VEGF agent (e.g., aflibercept, a functional fragment, or variant thereof) into an eye of a human subject.

[0130] Ocular disease or disorders that are approved for treatment with an anti-VEGF agent (e.g., bevacizumab, brotacizumab, ranibizumab, aflibercept, etc.) include, e.g., neovascular (wet) age-related macular degeneration (wAMD), macular edema following retinal vein occlusion (RVO), diabetic macular edema (DME) and diabetic retinopathy (DR) in patients with DME. In some embodiments, methods and pharmaceutical compositions disclosed herein are used to prevent or treat an ocular disease or disorder for which bevacizumab, brotacizumab, ranibizumab, and/or aflibercept is approved or indicated. In some embodiments, a gene therapy (e.g., AAV2.7m8 based gene therapy) is used to treat or prevent an ocular disease or disorder that is responsive to bevacizumab, brotacizumab, ranibizumab, and/or aflibercept, including, but not limited to, CNV, wet AMD, dry AMD, DME, RVO, macular edema following RVO, and diabetic retinopathy in patients with DME. In some embodiments, a rAAV gene therapy is used to treat or prevent any ocular disease or disorder characterized by neovascularization or CNV. In some embodiment, the methods and kits provided herein are for the treatment of diseases such as AMD, DME, RVO, angiogenesis related diseases, cancer, autoimmune diseases, infectious disease organisms, and the like.

[0131] In some embodiments, the ocular disease or disorder treated according to the methods described herein is diabetic macular edema. Diabetic macular edema (DME) is a swelling of the retina in diabetes mellitus due to leaking of fluid from blood vessels within the macula. The macula is the central portion of the retina, a small area rich in cones, the specialized nerve endings that detect color and upon which daytime vision depends. As macular edema develops, blurring occurs in the middle or just to the side of the central visual field. Visual loss from diabetic macular edema can progress over a period of months and make it impossible to focus clearly. Common symptoms of DME are blurry vision, floaters, double vision, and eventually blindness if it goes untreated. In some embodiments, methods and pharmaceutical compositions as disclosed herein are used to treat DME.

[0132] In some embodiments, the ocular disease or disorder treated according to the methods described herein is a retinal vein occlusion. Retinal vein occlusion is a blockage of the small veins that carry blood away from the retina. The retina is the layer of tissue at the back of the inner eye that converts light images to nerve signals and sends them to the brain. Retinal vein occlusion is most

often caused by hardening of the arteries (atherosclerosis) and the formation of a blood clot. Blockage of smaller veins (branch veins or BRVO) in the retina often occurs in places where retinal arteries that have been thickened or hardened by atherosclerosis cross over and place pressure on a retinal vein. Symptoms of retinal vein occlusion can include a sudden blurring or vision loss in all or part of one eye.

[0133] In some embodiments, the ocular disease or disorder treated according to the methods described herein is choroidal neovascularization (CNV), also known as wet age-related macular degeneration (wAMD). Choroidal neovascularization can involve the growth of new blood vessels that originate from the choroid through a break in the Bruch membrane into the sub-retinal pigment epithelium (sub-RPE) or subretinal space, which can be a major cause of visual loss. CNV can create a sudden deterioration of central vision, noticeable within a few weeks. Other symptoms can include color disturbances, and metamorphopsia (distortions in which straight lines appears wavy). Hemorrhaging of the new blood vessels can accelerate the onset of symptoms of CNV. CNV may also include feeling of pressure behind the eye.

[0134] 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. 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. In some embodiments, methods and pharmaceutical compositions as disclosed herein are used to treat AMD.

[0135] In some embodiments, methods described herein are used to prevent or treat an ocular disease or disorder that is responsive to treatment with bevacizumab, brolucizumab, ranibizumab, and/or aflibercept. In some embodiments, methods described herein are used to prevent or treat an ocular disease or disorder in a subject who has received prior treatment with bevacizumab, brolucizumab, ranibizumab, and/or aflibercept.

[0136] In some embodiments, methods and pharmaceutical compositions disclosed herein, i.e., AAV gene therapy comprising an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof), results in a reduction in neovascularization or CNV, as measured by percentage of grade IV lesions following CNV formation according to color fundus photography, by at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 25%, at least 30%, at least 35%, at least

40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 100% as compared to a vehicle or buffer control.

[0137] In some embodiments, methods and pharmaceutical compositions disclosed herein, i.e., AAV gene therapy comprising an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof) results in a reduction in neovascularization or CNV, as measured by percentage of grade IV lesions following CNV formation according to color fundus photography, that is comparable to, e.g., an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof) or a non-gene therapy-based anti-VEGF agent. In some embodiments, the reduction in CNV, or the therapeutic effect, lasts longer with the administration of a gene therapy comprising an anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof) as compared to administration with a non-gene therapy-based anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof) or a protein solution of the anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof). In some embodiments, the therapeutic effect of anti-VEGF gene therapy (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof) lasts for at least 1 year, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more years after a single intravitreal injection. In some embodiments, pharmaceutical compositions disclosed herein inhibit or sequester endogenous VEGF and/or PIGF.

Pharmaceutical Compositions

[0138] Provided herein are pharmaceutical compositions (such as pharmaceutical formulations) comprising one or more active ingredients, e.g., an AAV2.7m8 vector that comprises a nucleic acid sequence that encodes the anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof) as well as one or more excipients, carriers, stabilizers, or bulking agents. The pharmaceutical compositions are suitable for administration to a human patient via intravitreal (IVT) injection to achieve a desired therapeutic or prophylactic effect.

[0139] In some embodiments, the pharmaceutical composition comprising, e.g., an AAV2.7m8 vector that comprises a nucleic acid sequence that encodes the anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof), is supplied as a reconstituted homogenous solution. In some embodiments, the solution is a suspension. In some embodiments, the solution is isotonic. In other embodiments, the pharmaceutical composition comprising e.g., an AAV2.7m8 vector that comprises a nucleic acid sequence that encodes the anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof), is supplied in a lyophilized form, and is reconstituted prior to

administration to a patient. In some embodiments, the methods provided herein further comprise the steps of reconstituting, dissolving, or solubilizing a lyophilized pharmaceutical composition comprising rAAV (e.g., AAV2.7m8) and encodes anti-VEGF agent (e.g., bevacizumab, brolucizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof) in a buffer prior to administration to the subject. In some embodiments, such lyophilized pharmaceutical composition comprises one or more of the following: a cryoprotectant, a surfactant, a salt, a stabilizer, or any combination thereof.

[0140] In some embodiments, the pharmaceutical composition is a homogenous solution. In some embodiments, the homogenous solution is supplied in a pre-filled syringe. In some embodiments, the pharmaceutical composition is supplied as a suspension. In some embodiments, a suspension is a solution. In some embodiments, the suspension is refrigerated. In some embodiments, method provided herein further comprise the step of warming the refrigerated suspension to room temperature and/or agitating the suspension to ensure that the active ingredient(s) are dissolved and/or evenly distributed in solution prior to administering the pharmaceutical to the subject (e.g., via IVT injection). In some embodiments, the suspension is diluted prior to administration to the subject (e.g., via IVT injection). In some embodiments, the suspension is supplied as a pre-filled syringe.

[0141] In some embodiments, the pharmaceutical composition is provided as a refrigerated suspension. In some embodiments, the suspension comprises a pharmaceutically acceptable excipient, e.g., surfactant, glycerol, non-ionic surfactant, buffer, glycol, salt, and any combination thereof. In some embodiments, hydrochloric acid and sodium hydroxide are used to adjust the pH of the solution. In some embodiments, the refrigerated suspension is at a neutral pH, or at a pH between about 6.5 and about 7.5. In some embodiments, the pH of the refrigerated suspension is slightly basic (e.g., having a pH of about any one of 7.5, 8, 8.2, 8.4, 8.5, or 9, including any range in between these values). In some embodiments, the pH of the suspension or solution is slightly acidic (e.g., having a pH of about 6.5, 6.3, 6.1, 6, 5.5, or 5, including any range in between these values). In some embodiments, the suspension is a solution. In some embodiments, the suspension comprises micelles. In some embodiments, suspension is agitated and/or warmed to room temperature before administration to the subject (e.g., via IVT injection).

[0142] Also provided herein are kits comprising at least one pharmaceutical composition described herein. In some embodiments, the kit comprises a comprising lyophilized or freeze-dried pharmaceutical composition (e.g., one unit dose in a vial) disclosed herein and a solution for dissolving, diluting, and/or reconstituting the lyophilized pharmaceutical composition. In some embodiments, the solution for reconstituting or dilution is supplied as a pre-filled syringe. In some embodiments, a kit comprises a freeze-dried or lyophilized pharmaceutical composition comprising rAAV (e.g., AAV2.7m8) and a solution for reconstituting the pharmaceutical composition to a desired concentration or volume. In some embodiments, the kit includes a buffer that helps to prevent

aggregation upon reconstituting the pharmaceutical composition disclosed herein. In some embodiments, the pharmaceutical composition is provided in a pre-filled syringe. In some embodiments, a kit comprises a dual-chamber syringe or container wherein one of the chambers contains a buffer for dissolving or diluting the pharmaceutical composition. In some embodiments, the kit comprises a syringe for injection. In some embodiments, the reconstituted solution is filtered before administration. In some embodiments, the kit comprises a filter or a filter syringe for filtering the reconstituted pharmaceutical composition before administration to a patient.

[0143] In some embodiments, for storage stability and convenience of handling, a pharmaceutical composition, comprising rAAV (e.g., AAV2.7m8) and a nucleic acid sequence that encodes the anti-VEGF agent (e.g., bevacizumab, brovacizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof), is formulated as a lyophilized, freeze dried, or vacuum dried powder that is reconstituted with saline, buffer, or water prior to administration to a subject. Alternately, the pharmaceutical composition is formulated as an aqueous solution, such as a suspension or a homogeneous solution. A pharmaceutical composition can contain rAAV particles comprising a nucleic acid sequence that encodes aflibercept. In some embodiments, a different virus or delivery system, e.g., nanoparticles or lipid-based complexes, is used to deliver the nucleic acid sequence that encodes the anti-VEGF agent (e.g., bevacizumab, brovacizumab, ranibizumab, and/or aflibercept, or a functional fragment or variant thereof). Various excipients, such as phosphate, PBS, or Tris buffer, glycol, glycerol, saline, surfactant (e.g., pluronic or polysorbate), or any combination thereof, can be used to stabilize a pharmaceutical composition. Additionally, cryoprotectants, such as alcohols can be used as a stabilizer under freezing or drying conditions of lyophilization. In some embodiments, the gene therapy is provided as a suspension or a refrigerated suspension.

[0144] In some embodiments, a suspension or a reconstituted form of the lyophilized pharmaceutical composition comprising the anti-VEGF agent (e.g., aflibercept) gene therapy as disclosed herein has a volume of about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 μ L. In some embodiments, the suspension of the pharmaceutical composition comprising the anti-VEGF agent (e.g., aflibercept) gene therapy as disclosed herein has a volume of between 0.1 to 0.5 mL, between 0.1 to 0.2 mL, between 0.3 to 0.5 mL, between 0.5-1.0 mL, between 0.5-0.7 mL, between 0.6 to 0.8 mL, between 0.8 to 1 mL, between 0.9 to 1.1 mL, between 1.0 to 1.2 mL, or between 1.0 to 1.5 mL. In other embodiments, the volume is no more than 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, or 1.5 mL.

[0145] In some embodiments, pharmaceutical compositions disclosed herein are designed, engineered, or adapted for administration to a primate (e.g., non-human primate and human subjects) via intravitreal or subretinal injection. In some embodiments, a pharmaceutical composition comprising rAAV particles comprising a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept) is formulated for intravitreal injection into an eye of a subject. In some

embodiments, the pharmaceutical composition is formulated to or reconstituted to a concentration that allows intravitreal injection of a volume not more than about or not more than 2, 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 μ L. In some embodiments, a unit dose of the pharmaceutical composition comprises a volume not more than about or not more than 2, 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 μ L. In some embodiments, methods of treatment disclosed herein comprises intravitreal injection of a volume of about 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150 μ L of a solution comprising a rAAV (e.g., AAV2.7m8) and a nucleic acid sequence that encodes the anti-VEGF agent (e.g., aflibercept).

[0146] In some embodiments, an AAV2.7m8 particle comprising a nucleic acid sequence of the anti-VEGF agent (e.g., aflibercept) transgene described herein is a component of a gene therapy pharmaceutical composition. In some embodiments, a rAAV particle of any serotype comprising the 7m8 variant capsid protein as described herein is used to make a freeze-dried or lyophilized pharmaceutical composition or a suspension thereof. In some embodiments, the gene therapy is formulated as a refrigerated suspension. In some embodiments, the rAAV particle is rAAV2. In some embodiments, the lyophilized or suspension of the pharmaceutical composition comprises rAAV2 having the 7m8 variant capsid protein and a DNA sequence that encodes the anti-VEGF agent (e.g., aflibercept). In some embodiments, the suspension is refrigerated.

[0147] In some embodiments, the pharmaceutical composition is a unit dose (e.g., a therapeutically effective dose) to be administered to a subject (e.g., a human or non-human primate) via IVT injection for the treatment of an ocular disease or disorder characterized by abnormal (e.g., excessive) angiogenesis or neovascularization. In some embodiments, the pharmaceutical composition comprises a unit dose (e.g., a therapeutically effective dose) as described in further detail elsewhere herein. In some embodiments, the volume of the unit dose (e.g., a therapeutically effective dose) of a viral vector (e.g., an rAAV vector disclosed herein) administered to the subject is no more than about any one of about 50, 40, 30, 20, 10, or 5 μ L, including any range in between these values. Minimizing the volume of the unit dose to be administered to the subject may obviate or mitigate changes in ocular pressure and other adverse effects associated with IVT injection (e.g., elevated intraocular pressure, inflammation, irritation, or pain).

[0148] Pharmaceutical compositions suitable for ocular use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions, suspension, or dispersion. For intravitreal administration, suitable carriers include physiological saline, bacteriostatic water, phosphate buffered saline (PBS), and/or an isotonic agent, e.g., glycerol. In all embodiments, the pharmaceutical composition must be sterile and should be fluid to the extent that easy syringeability or injectability 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. In some embodiments, the pharmaceutical composition can include an isotonic agent, such as a salt or glycerol. In some embodiments, a surfactant or a stabilizer is added to the pharmaceutical composition to prevent aggregation.

[0149] In some embodiments, the excipient is a carrier. A carrier is a solvent or dispersion medium containing, for example, water, saline, ethanol, a polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and any combination 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 (e.g., Tween™, polysorbate 20, polysorbate 80), 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, cresol, thimerosal, and the like. In many embodiments, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, 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. In some embodiments, the pharmaceutical carrier includes sodium phosphate, sodium chloride, polysorbate, and sucrose. In some embodiments, a pharmaceutical composition comprises a surfactant, e.g., non-ionic surfactant such as polysorbate, poloxamer, or pluronic. In some embodiments, the addition of a non-ionic surfactant reduces aggregation in the pharmaceutical composition.

Articles of Manufacture and Kits

[0150] In some embodiments, provided are kits that comprise one or more pharmaceutical compositions disclosed herein for use according to a method described herein. In some embodiments, the kit comprises a recombinant viral vector (e.g., rAAV or rAAV2.7m8 comprising the nucleic acid sequence of the anti-VEGF agent (e.g., aflibercept)). In some aspects, the kit comprises a lyophilized form of a pharmaceutical composition and a solution for reconstituting the pharmaceutical composition prior to administration to a subject. In some embodiments, a kit comprises: a recombinant virus provided herein, and instructions to administer a unit dose of a pharmaceutical composition to a first eye the subject via intravitreal (IVT) injection at a first time point, and to administer a second unit dose of the pharmaceutical composition to a contralateral eye of the

subject via IVT injection at a second time point, according to any one of the methods described elsewhere herein.

[0151] In some embodiments, the kit comprises pharmaceutically acceptable excipients, buffers, solutions, etc. for administering the pharmaceutical composition. In some embodiments, the kit further comprises 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 unit dose (e.g., a therapeutically effective dose) of the pharmaceutical composition and/or to reconstitute lyophilized compositions. In some embodiments, the kit further comprises a device for administration, such as a syringe, filter needle, extension tubing, cannula, or other implements to facilitate intravitreal injection of the pharmaceutical composition.

[0152] In some embodiments, the kit comprises a pharmaceutical composition in the form of a suspension or refrigerated suspension, and a syringe and/or a buffer for dilution. In some embodiments, the kit comprises a pre-filled syringe comprising the suspension or refrigerated suspension.

[0153] The following description is presented to enable a person of ordinary skill in the art to make and use the various embodiments. Descriptions of specific devices, techniques, and applications are provided only as examples. Various modifications to the examples described herein will be readily apparent to those of ordinary skill in the art, and the general principles defined herein may be applied to other examples and applications without departing from the spirit and scope of the various embodiments. Thus, the various embodiments are not intended to be limited to the examples described herein and shown, but are to be accorded the scope consistent with the claims.

EXAMPLES

Example 1: Sequential Intravitreal Administration of AAV2.7m8-aflibercept Gene Therapy to the Contralateral Eyes in Non-Human Primates.

[0154] The ability of AAV vectors (e.g., AAV2.7m8) to efficiently transduce target retinal cells has been exploited to successfully transfer therapeutic genes into photoreceptors, retinal pigment epithelium, and the inner retina to treat a variety of retinal diseases. Intravitreal (IVT) AAV administration is a safe and convenient method of retinal delivery, but it has been suggested that neutralizing antibodies (nAb) against the vector capsid are more likely to be generated following IVT than following subretinal injection. Given that certain ocular diseases, such as wet age-related macular degeneration (wAMD), can affect both of an individual's eyes, there is a concern that nAb generated following IVT administration of an AAV to a first eye may decrease the efficiency of therapeutic gene transfer and prevent effective vector re-administration, e.g., to the individual's contralateral eye.

[0155] The experiments described below were conducted to assess the effect of prior exposure of AAV2.7m8-aflibercept in one eye on transduction efficacy of the same AAV vector in the contralateral eye of non-human primates (*i.e.*, St. Kitts African green monkeys). AAV2.7m8-aflibercept is a recombinant, replication-deficient adeno-associated virus (AAV.7m8) vector carrying a coding sequence for aflibercept.

[0156] Briefly, four monkeys were selected for the study. Prior to the beginning of the study, each monkey was examined and determined to have normal slit lamp and fundus exams, color fundus photographs (CFP), and optical coherence tomography (OCT). Additionally, each monkey was found to be negative for AAV.7m8 neutralizing antibodies (nAb) titers in an *in vitro* HEK293T cell-based assay prior to the study. On Day 0, three monkeys were administered with 2E12vg AAV2.7m8-aflibercept to the right eye (*i.e.*, OD) via intravitreal injection (IVT), and one monkey was administered with vehicle to both eyes (*i.e.*, OU). The volume of a human eye is approximately two times that of the African green monkey. Therefore, the dose administered to the monkeys (2E12 vg/eye) is equivalent to 4E12 vg/eye on a volume:volume basis. On Day 59, the three monkeys that had received AAV2.7m8-aflibercept OD were IVT administered with 2E12vg AAV2.7m8-aflibercept to the left eye (*i.e.*, OS). See *Table A* below.

Table A: Staggered Dosing of AAV2.7m8-Aflibercept in Contralateral Eyes

Treatment	N (number)	Dose (IVT)	Treatment delivery	Eye Treated	Follow up post-treatment
AAV2.7m8-aflibercept	3 monkeys	2x10 ¹² vg/100μL	Day 0	OD	264 Days (9 months)
		2x10 ¹² vg/100μL	Day 59	OS	205 Days (7 months)
Vehicle	1 monkey	100μL	Day 0	OU	264 Days (9 months)

[0157] Vitreous fluid and aqueous fluid samples were obtained from each of the four monkeys on Days 28, 56, 84, 112, 140, 168, 196, and 224 of the study and assessed via ELISA for aflibercept expression. Serum samples were collected in parallel and evaluated for the presence of neutralizing antibodies (nAb) that react with the 7m8 capsid using an *in vitro* HEK293T cell-based assay. Baseline samples of vitreous fluid and plasma were obtained prior to the study. Aflibercept expression in various ocular tissues was determined via ELISA post-mortem at study termination (*i.e.*, Day 264).

[0158] A time course of average aflibercept expression in the vitreous and aqueous fluids of the right eyes of the three monkeys that received AAV2.7m8-aflibercept on Day 0 of the study is shown

in **FIG. 1A**. The expression levels of aflibercept are consistent with historical data for the same dose. **FIG. 1B** shows a time course of average aflibercept expression in the vitreous and aqueous fluids of the left eyes (*i.e.*, the later dosed eyes) of the three monkeys that received AAV2.7m8-aflibercept on Day 59 of the study. Aflibercept expression levels in the left eyes (which received the later dose of AAV2.7m8-aflibercept) were about 3-fold lower than the expression levels in the right eyes at equivalent time points following IVT. Such expression level in the left eye is within the range that has been shown to provide therapeutic levels of aflibercept in a non-human primate model of choroidal neovascularization. **FIG. 1A** represents the average of the three expression levels shown in **FIGs. 2A** (vitreous) and **2B** (aqueous). **FIG. 1B** represents the average of the three expression levels shown in **FIGs. 3A** (vitreous) and **3B** (aqueous).

[0159] **FIG. 4** shows expression levels of aflibercept in the retina, choroid, and iris/ciliary body in each eye of each monkey at study termination (*i.e.*, on Day 264). In general, expression of aflibercept was highest in the retina. Aflibercept expression was found to be higher in the first eyes (*i.e.*, OD) of each monkey that was administered with AAV2.7m8-aflibercept via IVT.

[0160] There was an increase in post-IVT AAV2.7m8 nAb response in both the serum and vitreous fluids of the three monkeys administered with AAV2.7m8-aflibercept. *See FIGs. 5A and 5B*. Greater nAb responses were observed in the later-injected eyes (*i.e.*, OS). Serum nAb levels peaked about 1 month after the second eye injection in each monkey (*i.e.*, ~ Day 84), consistent with recall immune response. *Table B* below provides a qualitative summary of the data shown in **FIGs. 5A and 5B**.

Table B: Presence of anti-AAV2.7m8 immunoglobulin (IgG) in serum and vitreous samples at various time points

Sample		Animal ID	Treatment	Time Point									
				Baseline		Month 2		Month 4		Month 5		Month 9	
Serum		Monkey 1	AAV2.7m8	-		+		+		+		+	
		Monkey 2	AAV2.7m8	-		+		+		+		+	
		Monkey 3	AAV2.7m8	-		+		+		+		+	
		Control Monkey	Vehicle	-		-		-		-		-	
Vitreous Fluid	Vitreous Cytosol	Monkey 1	AAV2.7m8	-	-	+	-	+	+	+	+	+	+

	Monkey 2	AAV2.7m8	-	-	+	-	+	+	+	+	+	+
	Monkey 3	AAV2.7m8	-	-	+	-	+	+	+	+	+	+
	Control Monkey	Vehicle	-	-	-	-	-	-	-	-	-	-

[0161] Mild inflammation was observed in both eyes (data not shown), with second injection resulting in earlier onset likely due to recall immune response. Ocular health parameters were scored by the Hackett-McDonald irritation and inflammation scoring system (*see, e.g.*, Hackett, R. B. and McDonald, T. O. "Eye Irritation" in *Dermatotoxicology*, 4th edition, Marzulli, F. N. and Maibach, H. I. editors, Hemisphere Publishing Corp., Washington D.C. (1991)). FIG. 7 shows the levels of inflammatory keratic precipitate ("KP"), vitreous cell infiltrates, vitreous haze, and aqueous cell infiltrates detected, no aqueous flare was detected following IVT injections. (The arrows with dotted lines in FIG. 7 indicate time of injection.) Little to no vitreous haze was detected during slit lamp examination of the eye. Briefly, an aqueous flare is an abnormal appearance of the beam of light as it travels through the anterior chamber. The flare is caused by light reflecting off proteins in the aqueous humor and is typically found when there is inflammation in the anterior chamber. Aqueous and vitreous cell infiltrates were generally transient and self-resolving. The ophthalmic effects summarized in FIG. 7 indicate that bilateral IVT administration AAV2.7m8 was well-tolerated with no serious adverse effects.

[0162] Increase in retinal volume and/or thickening of the retina is indicative of edema, whereas retinal thinning is indicative of loss of retinal cells. Retinal thickness and retinal volume of monkeys that received bilateral IVT administration were as assessed via optical coherence tomography (OCT). As shown in FIG. 8, there was little to no difference in the retinal thickness or retinal volumes of the right eyes (which received the first IVT injections) and left eyes (which received the later IVT injections) in monkeys treated with AAV2.7m8. Moreover, there was little to no difference in the retinal thickness or retinal volumes of monkeys receiving bilateral IVT doses of AAV2.7m8 as compared to monkeys receiving vehicle.

[0163] Retinal tissue sections were stained with hematoxylin (which stains nucleic acids) and eosin (which stains proteins) to assess retinal morphology and determine whether cell death occurred and/or whether immune cells infiltrated the retina. FIG. 9 and in Table C below indicate that lesions and perivascular infiltrates were minimal. FIG. 9 also shows that bilateral IVT administration of AAV2.7m8 did not elicit strong immune responses in the monkeys' left (i.e., later-dosed) eyes.

Table C: Histopathology Assessment of Retinal Tissues

Tissue and Finding	Vehicle		7m8.CO.Aflibercept					
Animal ID	Control Monkey		Monkey 1		Monkey 2		Monkey 3	
Eye	1 st (OD)	2 nd (OS)	1 st (OD)	2 nd (OS)	1 st (OD)	2 nd (OS)	1 st (OD)	2 nd (OS)
Grade	NVL	NVL	1	1	1	1	1	1

Histopathology scoring scale: NVL = no visible lesions; Grade 1 = minimal; Grade 2 = mild; Grade 3 = moderate; Grade 4 = marked; Grade 5 = severe.

[0164] The data discussed above demonstrated that development of immunity following IVT administration of AAV2.7m8 capsid in one eye does not completely block transduction following sequential dosing in the contralateral eye. Staggered IVT dosing to both eyes with AAV2.7m8-aflibercept was well-tolerated with minimal perivascular infiltrates and mild inflammation.

[0165] Although the present disclosure has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the present disclosure. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

CLAIMS

1. A method of treating an ocular disease or disorder in a subject, comprising:
 - (i) administering a first unit dose of a pharmaceutical composition to a first eye of the subject via intravitreal (IVT) injection at a first time point, and
 - (ii) administering a second unit dose of the pharmaceutical composition to a contralateral eye of the subject via IVT injection at a second time point,wherein the pharmaceutical composition comprises:
 - (a) a recombinant adeno-associated virus (rAAV) particle comprising a nucleic acid encoding an anti-vascular endothelial growth factor (VEGF) agent, wherein the rAAV particle is capable of infecting a retinal cell following IVT injection and
 - (b) a pharmaceutically acceptable excipient.
2. The method of claim 1, wherein the method further comprises a step of measuring a level of neutralizing antibodies against the rAAV in a sample from the subject following the first time point and prior to the second time point.
3. The method of claim 1 or 2, wherein the method further comprises a step of measuring expression level of the nucleic acid encoding the anti-vascular endothelial growth factor (VEGF) agent in a sample from the subject following the first time point and prior to the second time point.
4. The method of any one of claims 1-3, wherein the time interval between the first time point and the second time point is at least about 2 weeks.
5. The method of any one of claims 1-3, wherein the time interval between the first time point and the second time point at least about 4 weeks or about 1 month.
6. The method of any one of claims 1-3, wherein the time interval between the first time point and the second time point is at least about 6 weeks.
7. The method of any one of claims 1-3, wherein the time interval between the first time point and the second time point is at least about 8 weeks.
8. The method of any one of claims 1-7, wherein the first unit dose and the second unit dose each comprise between about 1E9 and about 3E13 vector genomes.

9. The method of any one of claims 1-8, wherein the first unit dose and the second unit dose each comprise between about $1E10$ and about $3E12$ vector genomes.
10. The method of any one of claims 1-8, wherein the first unit dose and the second unit dose each comprise between $1E11$ and $1E13$ vector genomes.
11. The method of claim 10, wherein the first unit dose and the second unit dose each comprise between $2E11$ and $6E12$ vector genomes.
12. The method of any one of claims 1-11, wherein the second unit dose is higher than the first unit dose.
13. The method of claim 12, wherein the second unit dose is at least about 300% of the first unit dose.
14. The method of claim 12 or 13, wherein the second unit dose is between about 300% and about 1000% of the first unit dose.
15. The method of any one of claims 12-14, wherein the first unit dose comprises about $6E10$ vector genomes the second unit dose comprises between about $1.8E11$ and about $6E11$ vector genomes.
16. The method of any one of claims 12-14, wherein the first unit dose comprises about $6E11$ vector genomes and the second unit dose comprises between about $1.8E12$ and about $6E12$ vector genomes.
17. The method of any one of claims 12-14, wherein the first unit dose comprises about $2E11$ vector genomes and the second unit dose comprises between about $6E11$ and $2E12$ about vector genomes.
18. The method of any one of claims 12-14, wherein the first unit dose comprises about $2E12$ vector genomes and the second unit dose comprises between about $6E12$ and about $2E13$ vector genomes.
19. The method of any one of claims 1-18, wherein the volumes of first unit dose and the second unit dose are each no more than about $100\ \mu\text{L}$.

20. The method of claim 17, wherein the volumes of first unit dose and the second unit dose are each no more than about 50 μ L.
21. A method treating an ocular disease or disorder in a subject, comprising:
administering a unit dose of a pharmaceutical composition to one eye of the subject via intravitreal (IVT) injection,
wherein the pharmaceutical composition comprises:
(a) a recombinant adeno-associated virus (rAAV) particle comprising a nucleic acid encoding an anti-vascular endothelial growth factor (VEGF) agent, wherein the rAAV particle is capable of infecting a retinal cell following IVT injection, and
(b) a pharmaceutically acceptable excipient, and
wherein the subject was administered with a prior unit dose of the pharmaceutical composition to a contralateral eye via IVT injection.
22. The method of claim 21, wherein the method further comprises a step of measuring a level of neutralizing antibodies against the rAAV in a sample from the subject following administration of the prior unit dose to the contralateral eye and prior to the administration of the unit dose to the one eye.
23. The method of claim 21 or 22, wherein the method further comprises a step of measuring expression level of the nucleic acid encoding the anti-vascular endothelial growth factor (VEGF) agent in a sample from the subject following administration of the prior unit dose to the contralateral eye and prior to the administration of the unit dose to the one eye.
24. The method of any one of claims 21-23, wherein the unit dose comprises between $1\text{E}9$ to $3\text{E}13$ vector genomes.
25. The method of any one of claims 21-24, wherein the unit dose comprises between $1\text{E}10$ to $3\text{E}12$ vector genomes.
26. The method of any one of claims 21-24, wherein the unit dose comprises between $1\text{E}11$ and $1\text{E}13$ vector genomes.
27. The method of claim 26, wherein the unit dose comprises between $2\text{E}11$ and $6\text{E}12$ vector genomes.

28. The method of any one of claims 21-27, wherein the prior unit dose comprised between 1E9 to 3E13 vector genomes.
29. The method of any one of claims 21-28, wherein the prior unit dose comprised between 1E10 to 3E12 vector genomes.
30. The method of any one of claim 21-28, wherein the prior unit dose comprised between 1E11 and 1E13 vector genomes.
31. The method of claim 30, wherein the prior unit dose comprised between 2E11 and 6E12 vector genomes.
32. The method of any one of claims 21-29, wherein the unit dose administered to the one eye is higher than the prior unit dose that was administered to the contralateral eye.
33. The method of claim 32, wherein the unit dose is at least about 300% of the prior unit dose.
34. The method of claim 32 or 33, wherein the unit dose is between about 300% and about 1000% of the prior unit dose.
35. The method of any one of claims 32-34, wherein the prior unit dose comprised about 6E10 vector genomes and the unit dose comprises between about 1.8E11 and about 6E11 vector genomes.
36. The method of any one of claims 32-34, wherein the prior unit dose comprised about 6E11 vector genomes and the unit dose comprises between about 1.8E12 and about 6E12 vector genomes.
37. The method of any one of claims 32-34, wherein the prior unit dose comprised about 2E11 vector genomes and the unit dose comprises between about 6E11 and 2E12 about vector genomes.
38. The method of any one of claims 32-34, wherein the prior unit dose comprised about 2E12 vector genomes and the unit dose comprises between about 6E12 and about 2E13 vector genomes.

39. The method of any one of claims 21-38, wherein the time interval between the administration of the prior unit dose and administration of the unit dose is at least about 2 weeks.
40. The method of any one of claims 21-38, wherein the time interval between the administration of the prior unit dose and administration of the unit dose is at least about 4 weeks or about 1 month.
41. The method of any one of claims 21-38, wherein the time interval between the administration of the prior unit dose and administration of the unit dose is at least about 6 weeks.
42. The method of any one of claims 21-38, wherein the time interval between the administration of the prior unit dose and administration of the unit dose is at least about 8 weeks or about two months.
43. The method of any one of claims 1-42, wherein the rAAV particle comprises a variant capsid protein that comprises a peptide insertion relative to a corresponding parental AAV capsid protein, wherein the peptide insertion has an amino acid sequence selected from LALGETTRPA (SEQ ID NO: 1); LANETITRPA (SEQ ID NO: 2), LAKAGQANNA (SEQ ID NO: 3), LAKDPKTTNA (SEQ ID NO: 4), KDTDTTR (SEQ ID NO: 5), RAGGSVG (SEQ ID NO: 6), AVDTTKF (SEQ ID NO: 7), STGKVPN (SEQ ID NO: 8), LAKDTDTTRA (SEQ ID NO: 9), LARAGGSVGA (SEQ ID NO: 10), LAAVDTTKFA (SEQ ID NO: 11), and LASTGKVPNA (SEQ ID NO: 12), wherein the insertion site is located between two adjacent amino acids at a position between amino acids corresponding to amino acids 570 and 611 of VP1 of AAV2 or the corresponding position in the capsid protein of another AAV serotype.
44. The method of claim 43, wherein the rAAV particle is an rAAV2 particle that comprises a variant capsid protein that comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 1) inserted between positions 587 and 588 of SEQ ID NO: 13.
45. The method of claim 44, wherein the variant capsid protein comprises the amino acid sequence of SEQ ID NO: 46.
46. The method of any one of claims 1-42, wherein the rAAV particle comprises a variant capsid protein that comprises a modified sequence, the modified sequence comprising one or more amino acid substitutions within amino acid residues 570-579 relative to a parental AAV capsid protein, wherein the modified sequence comprises HKFKSGD (SEQ ID NO: 37), and wherein the amino acid residue numbering corresponds to an AAV5 VP1 capsid protein.

47. The method of claim 46, wherein the parental AAV capsid protein is an AAV5 capsid protein or an AAV5 and AAV2 hybrid capsid protein.
48. The method of claim 46 or 47, wherein the parental AAV capsid protein is a AAV2.5T capsid protein.
49. The method of any one of claims 46-48, wherein the parental AAV capsid protein is an AAV2.5T VP1 capsid protein.
50. The method of any one of claims 46-49, wherein the modified sequence comprises LAHKFKSGDA (SEQ ID NO: 39).
51. The method of any one of claims 46-50, wherein the variant AAV capsid protein comprises a capsid sequence having at least 85% homology to the amino acid sequence set forth in SEQ ID NO: 40 or SEQ ID NO: 41.
52. The method of any one of claims 46-51, wherein the variant AAV capsid protein comprises a capsid sequence set forth in SEQ ID NO: 42 or SEQ ID NO:43.
53. The method of any one of claims 1-52, wherein the anti-VEGF agent is a bevacizumab, brolicizumab, or ranibizumab.
54. The method of any one of claims 1-53, wherein the anti-VEGF agent is a polypeptide that comprises an amino acid sequence having at least 80% homology to aflibercept.
55. The method of claim 54, wherein the anti-VEGF agent is aflibercept.
56. The method of any one of claims 1-55, wherein the retinal cell is a photoreceptor, a retinal ganglion cell, a Müller cell, a bipolar cell, an amacrine cell, a horizontal cell, or a retinal pigmented epithelium cell.
57. The method of claim any one of claims 1-56, wherein the ocular disease or disorder is choroidal neovascularization, wet age-related macular degeneration (wAMD), macular edema following retinal vein occlusion, diabetic macular edema (DME), or diabetic retinopathy associated with DME.

58. The method of claim 57, wherein the ocular disease or disorder is choroidal neovascularization or wet AMD.
59. The method of any one of claims 1-58, wherein the subject is a human.
60. The method of any one of claims 1-58, wherein the subject is responsive to administration of an anti-VEGF agent, wherein the anti-VEGF agent is a polypeptide.
61. The method of claim 60, wherein the anti-VEGF agent is aflibercept.
62. The method of any one of claims 1-61, wherein the subject received prior treatment for the ocular disease or disorder with an anti-VEGF agent.
63. The method of claim 62, wherein the anti-VEGF agent was aflibercept.

FIG. 1A

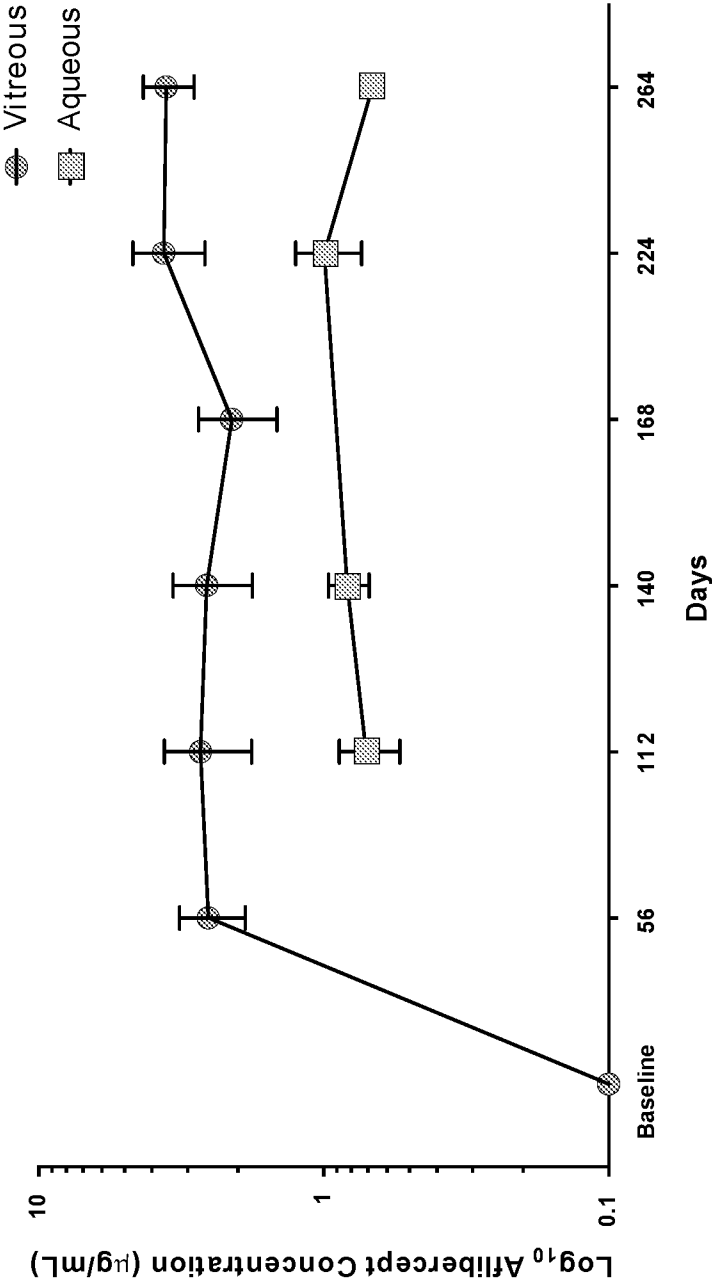
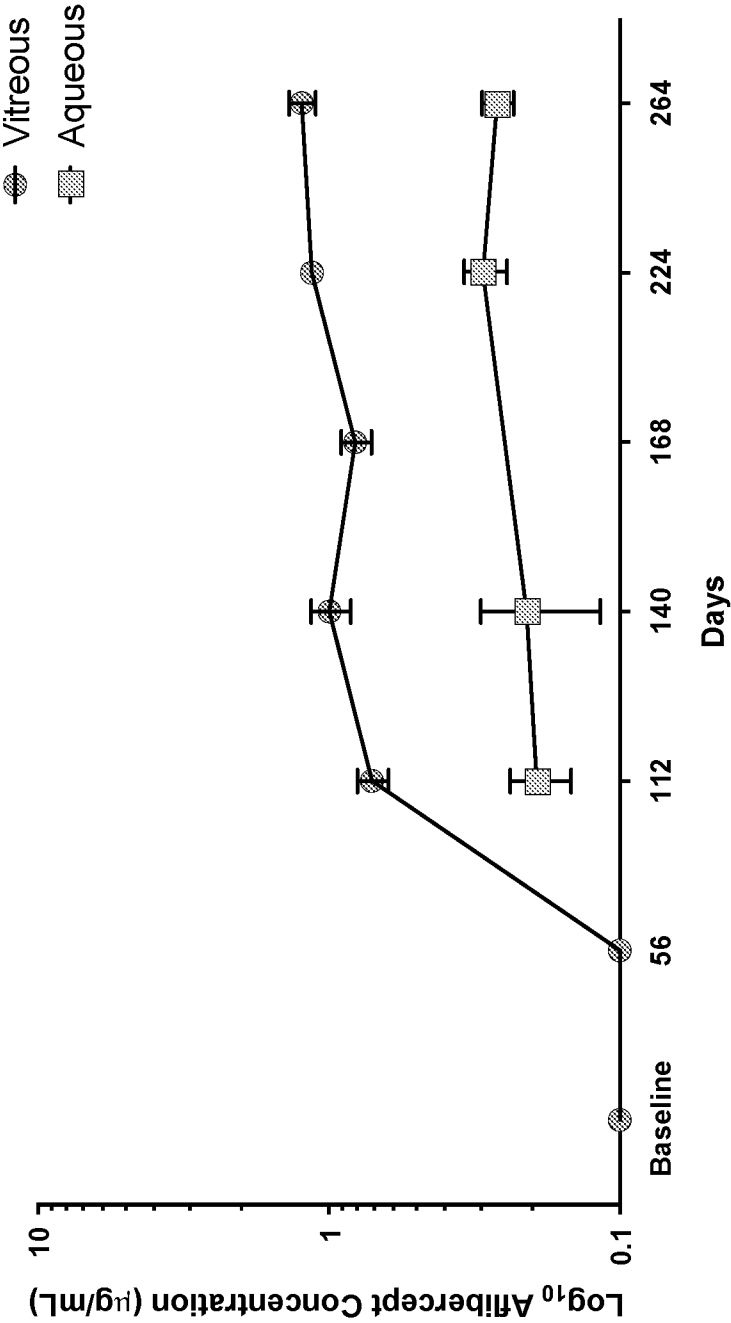


FIG. 1B



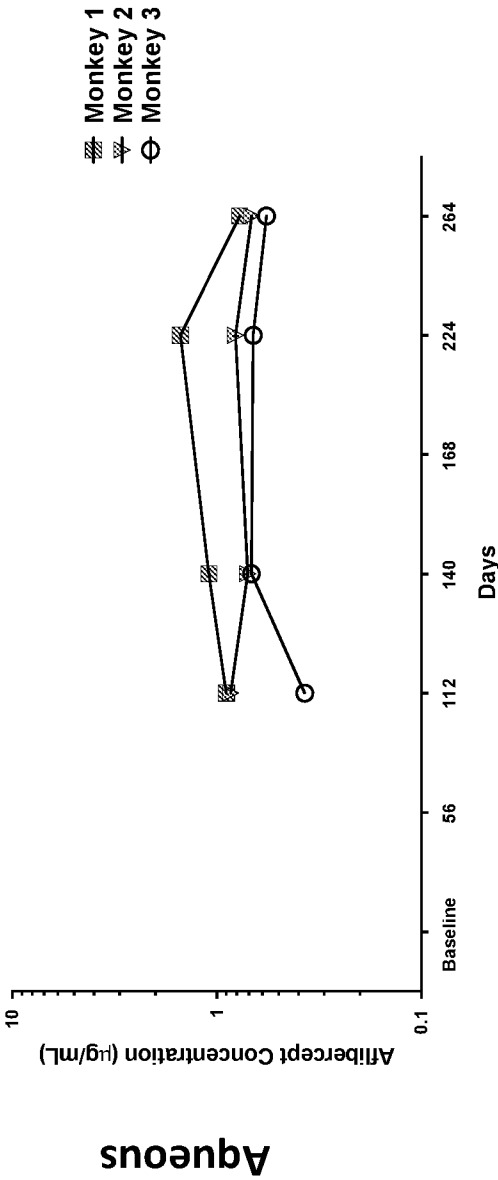
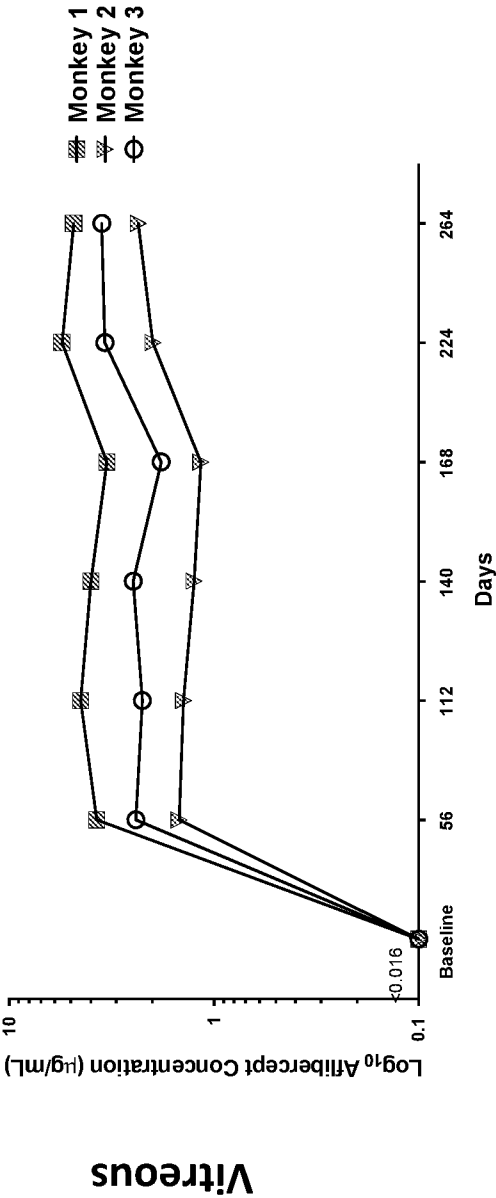
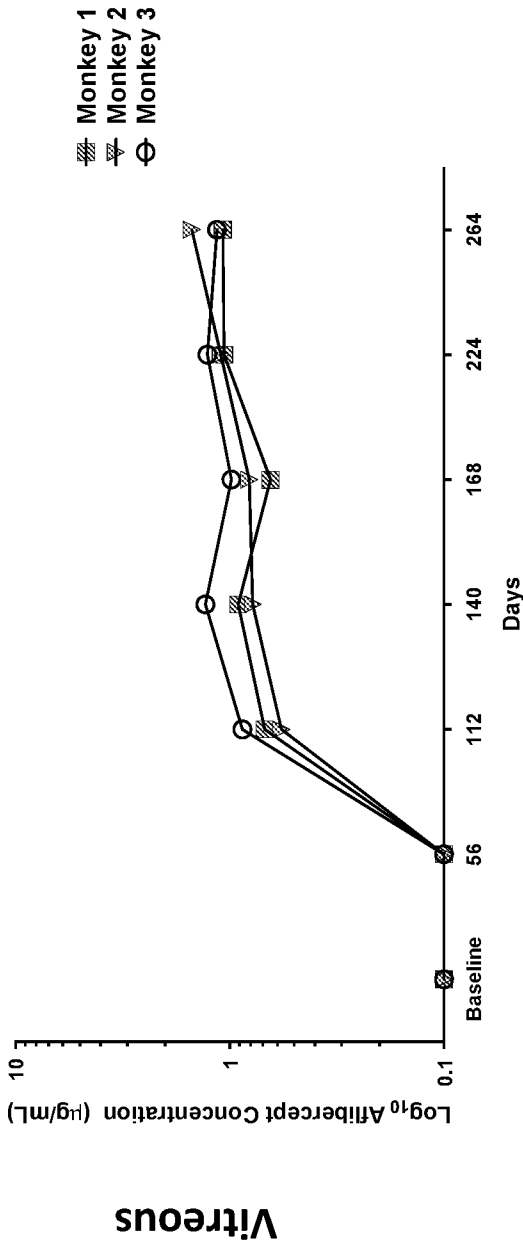


FIG. 3A



Aqueous

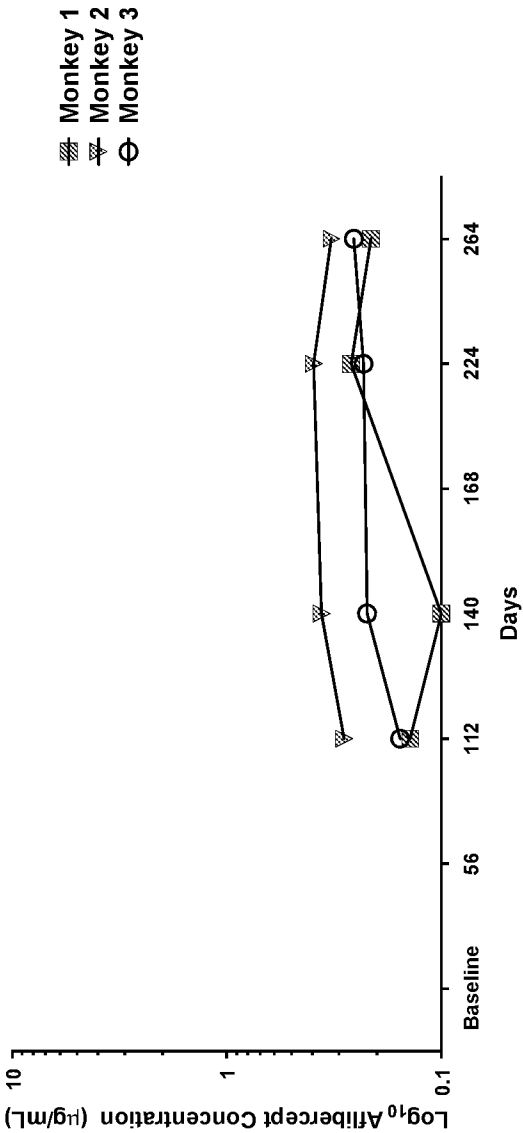


FIG. 4

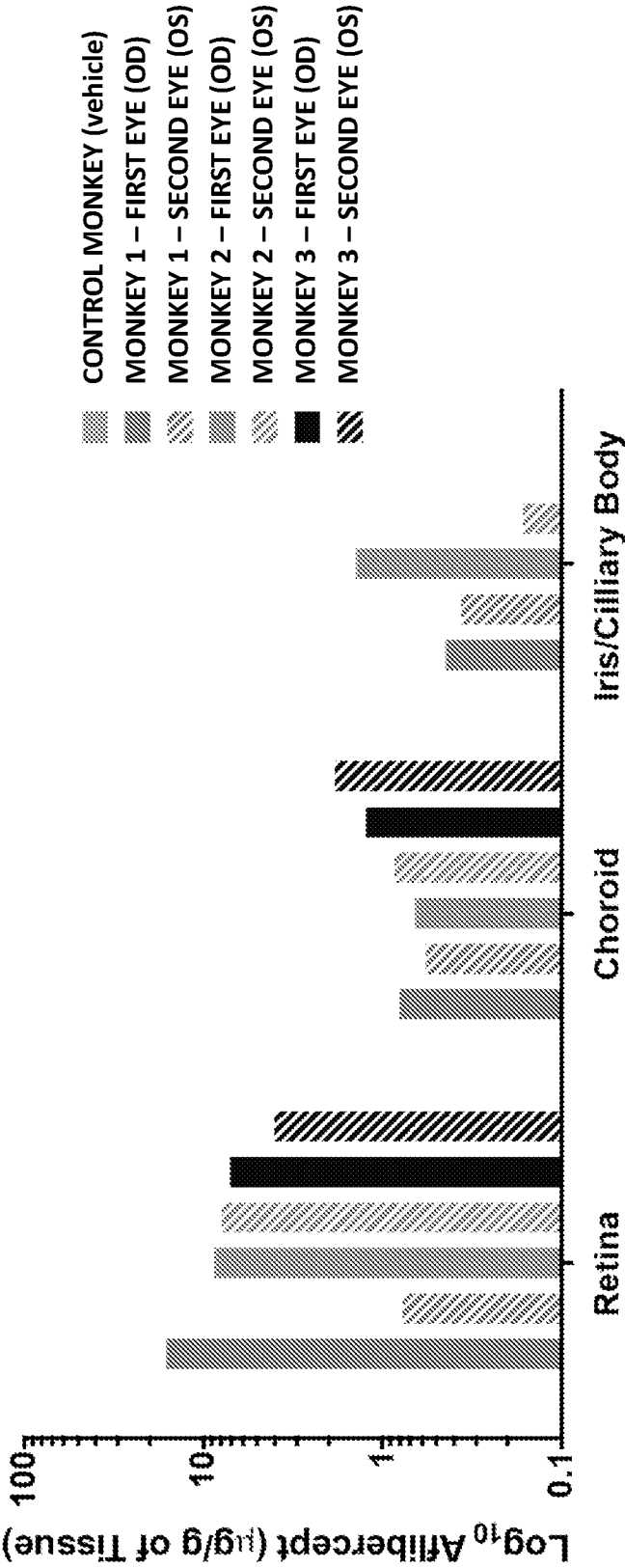


FIG. 5A

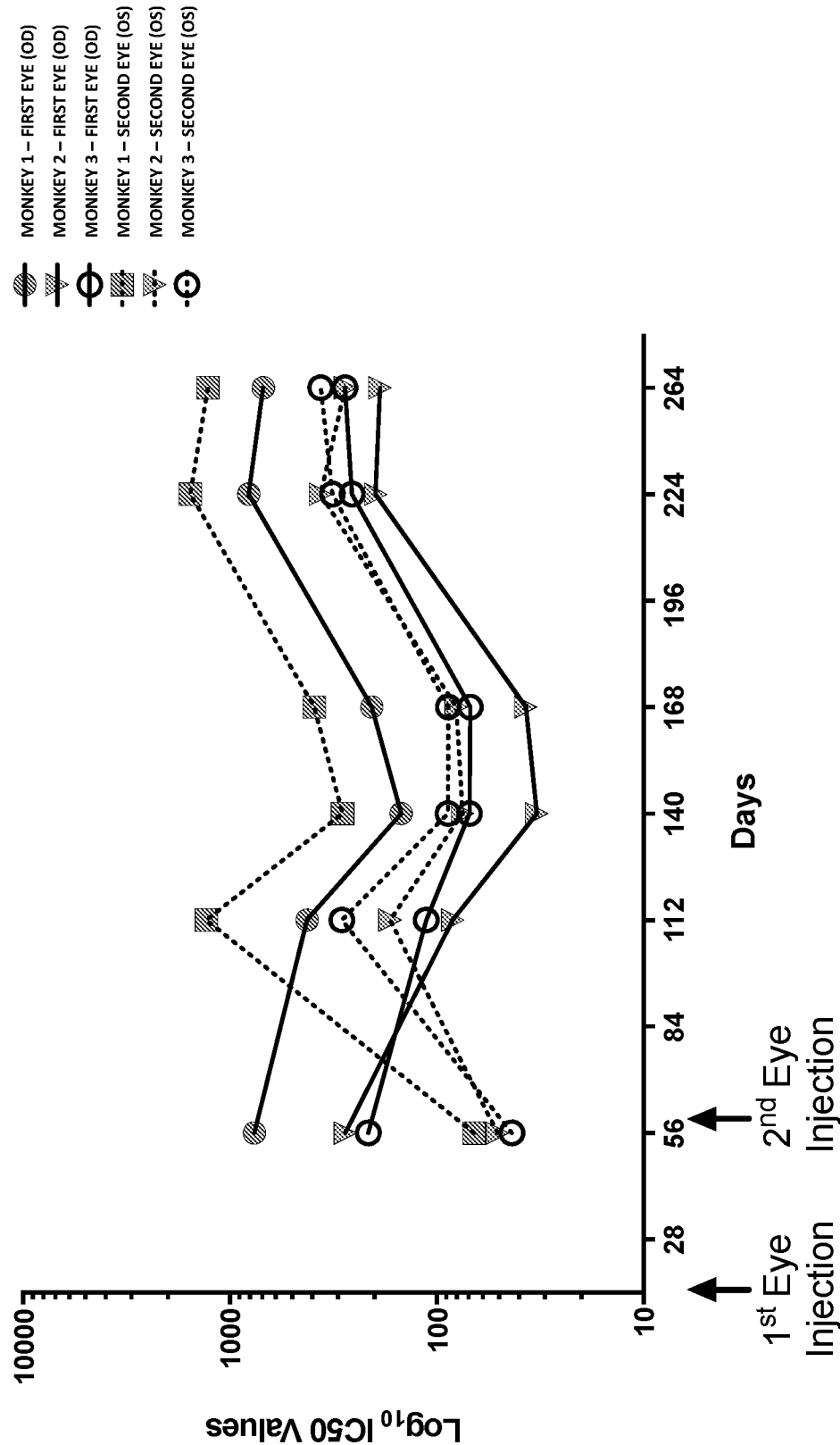


FIG. 5B

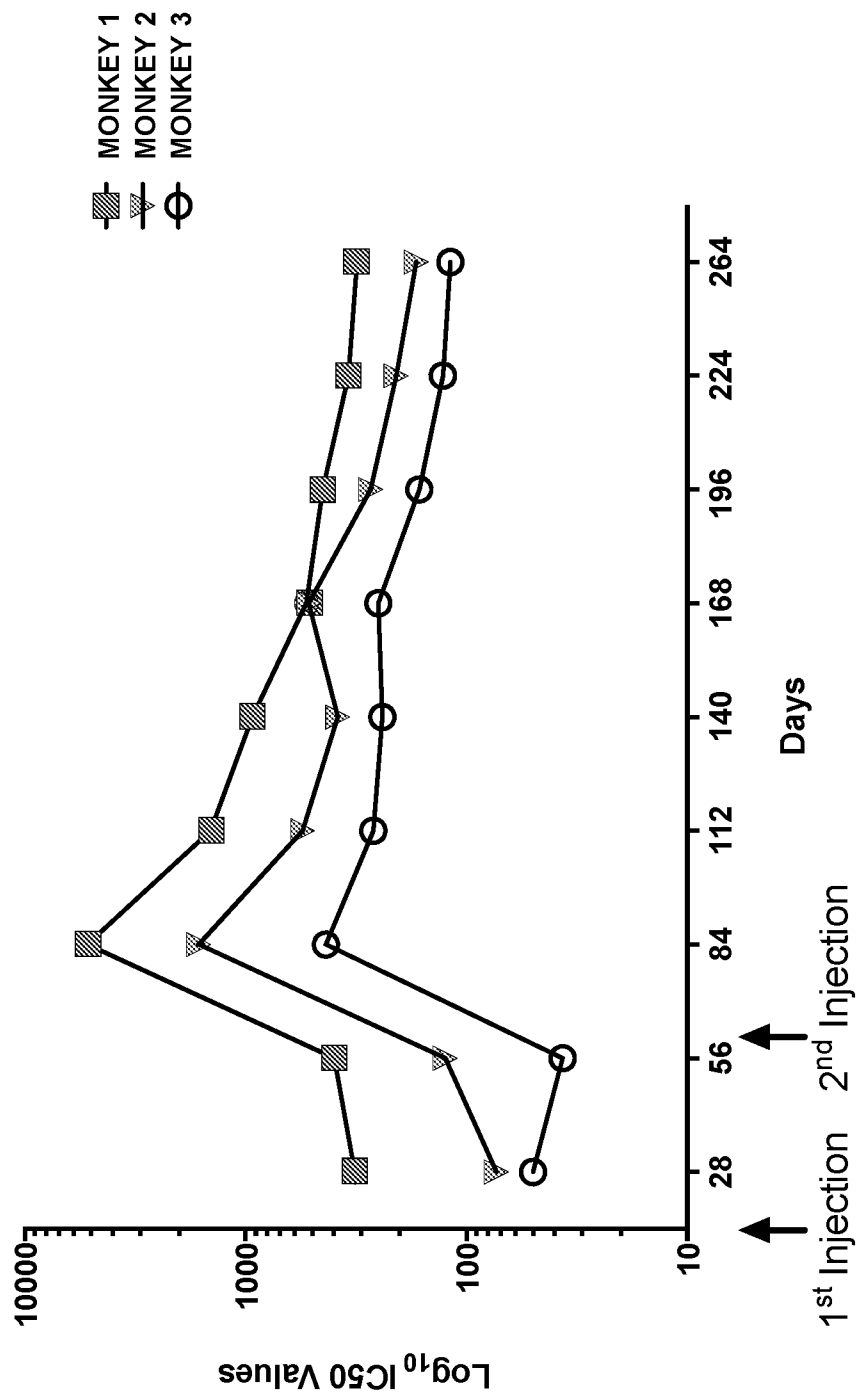


FIG. 6

ATGGTCAGCTACTGGGACACCGGGTCCTGCTGTGTGGCGCTGCTCAGCTGTCTGC
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 CAGTGAAATCCCCGAAATTATACACATGACTGAAGGAAGGAGCTCGTCATTCC
 CTGCCGGGTACGTCACTTAACATCACTGTTACTTTAAAAAAGTTTCCACTTGAC
 ACTTTGATCCCTGATGGAAAACCGCATATCTGGGACAGTAGAAAAGGGCTTCATC
 ATATCAAAATGCAACGTACAAAAGAAATAGGGCTTCTGACCTGTGGAAGCAACAGTC
 AATGGGCATTTGTATAGACAAAACATACTACACACATCGACAAACCAATACAATC
 ATAGATGTGGTTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTGGAGAAAAGC
 TTGTCTTAAATTGTACAGCAAGAACTGAACATAATGTGGGATTGACTTCAACTG
 GGAATACCCCTTCTCGAAGCATCAGCATAGAAACTTGTATAACCGAGACCTAAA
 AACCCAGTCTGGGAGTGAGATGAAGAAATTTTGAGCACCTTAACATAAGATGG
 TGTAAACCCGAGTGACCAAGGATTTGTACACCTGTGCAGCATCCAGTGGGCTGAT
 GACCAAGAAAGAACAGCACATTTGTACAGGGTCCATGAAAAGGACAAAACCTCACA
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 CCCCCAAAACCAAGGACACCTCATGATCTCCCGACCCCTGAGGTCACATG
 CGTGGTGGTGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGT
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 TTCTCATGCTCCGTGATGCAATGAGGCTCTGCACAACCACTACACGCAAGAGAGC
 CTCCTCCCTGCTCCGGGTAAATGA

FIG. 7

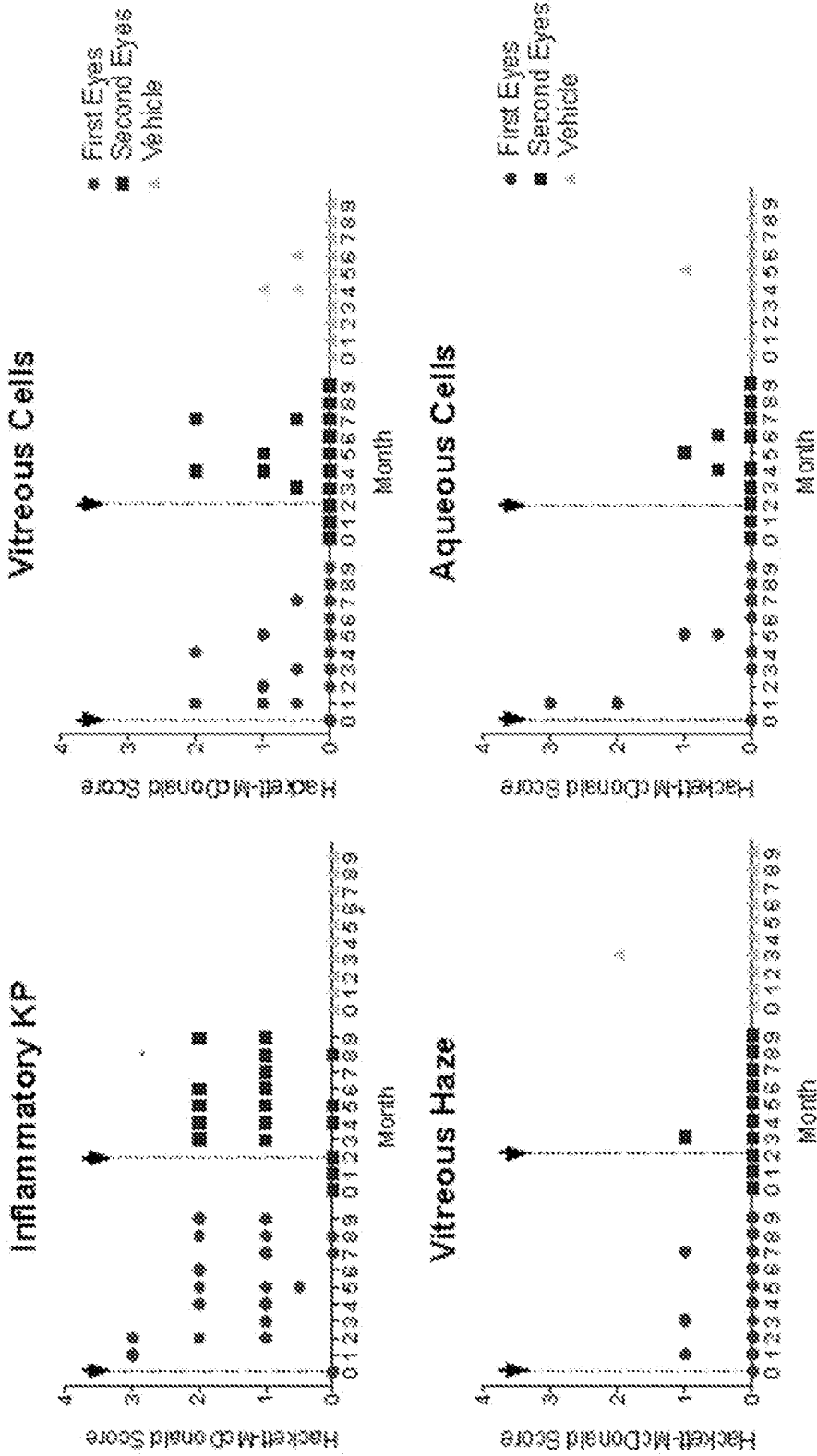


FIG. 8

OCT

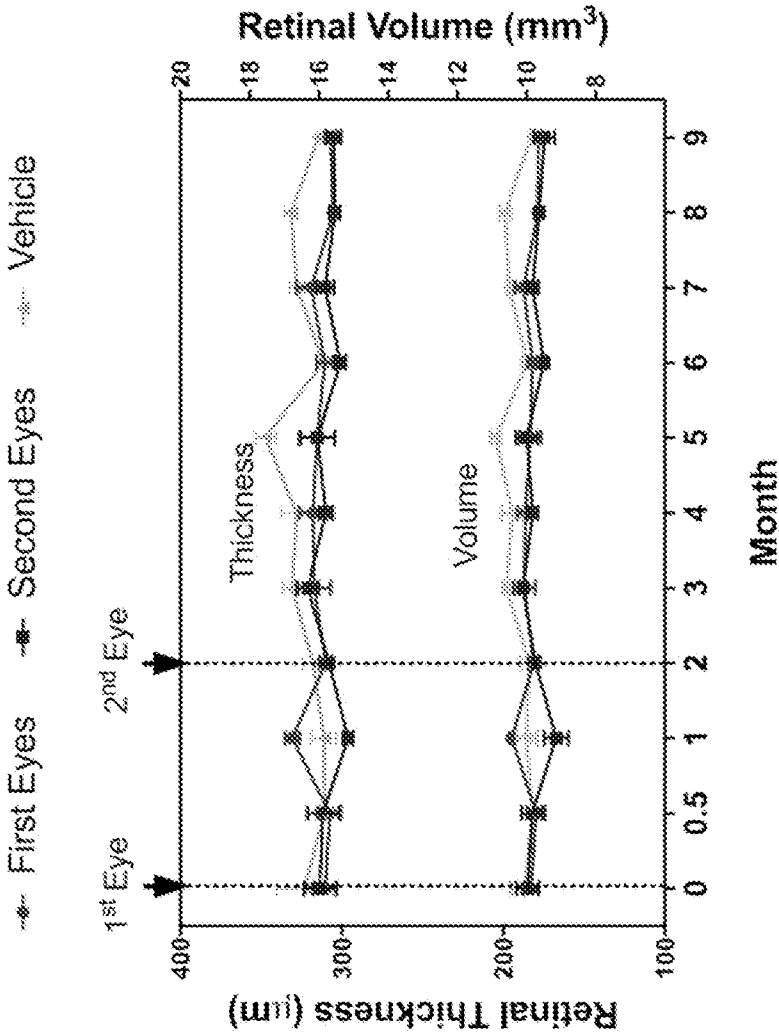
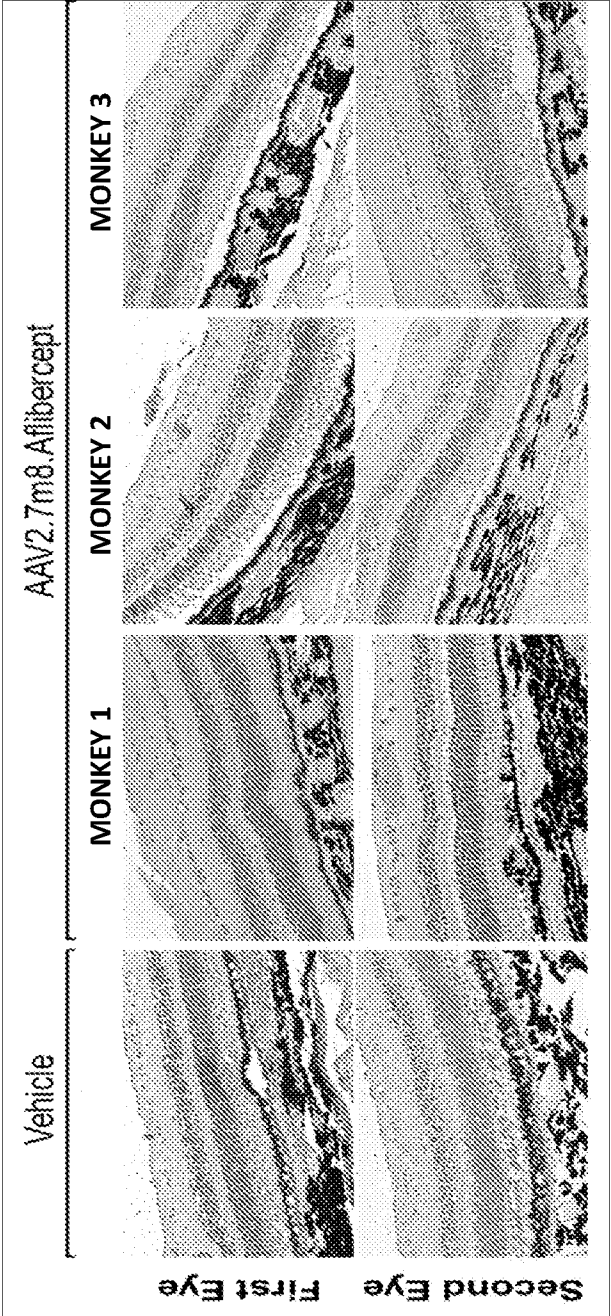


FIG. 9



SEQUENCE LISTING

<110> Adverum Biotechnologies, Inc.

<120> SEQUENTIAL INTRAVITREAL ADMINISTRATION OF AAV GENE
THERAPY TO CONTRALATERAL EYES

<130> 62700-20010.40

<140> Not Yet Assigned

<141> Concurrently Herewith

<150> US 62/839,457

<151> 2019-04-26

<150> US 62/813,597

<151> 2019-03-04

<160> 46

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 1

Leu Ala Leu Gly Glu Thr Thr Arg Pro Ala

1 5 10

<210> 2

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 2

Leu Ala Asn Glu Thr Ile Thr Arg Pro Ala

1 5 10

<210> 3

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 3

Leu Ala Lys Ala Gly Gln Ala Asn Asn Ala

1 5 10

<210> 4

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 4

Leu Ala Lys Asp Pro Lys Thr Thr Asn Ala
1 5 10

<210> 5

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 5

Lys Asp Thr Asp Thr Thr Arg
1 5

<210> 6

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 6

Arg Ala Gly Gly Ser Val Gly
1 5

<210> 7

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 7

Ala Val Asp Thr Thr Lys Phe
1 5

<210> 8

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 8

Ser Thr Gly Lys Val Pro Asn
1 5

<210> 9

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 9

Leu Ala Lys Asp Thr Asp Thr Thr Arg Ala

<400> 13																
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1				5					10					15		
Glu	Gly	Ile	Arg	Gln	Trp	Trp	Lys	Leu	Lys	Pro	Gly	Pro	Pro	Pro	Pro	
			20					25					30			
Lys	Pro	Ala	Glu	Arg	His	Lys	Asp	Asp	Ser	Arg	Gly	Leu	Val	Leu	Pro	
		35					40					45				
Gly	Tyr	Lys	Tyr	Leu	Gly	Pro	Phe	Asn	Gly	Leu	Asp	Lys	Gly	Glu	Pro	
	50					55					60					
Val	Asn	Glu	Ala	Asp	Ala	Ala	Ala	Leu	Glu	His	Asp	Lys	Ala	Tyr	Asp	
65				70						75				80		
Arg	Gln	Leu	Asp	Ser	Gly	Asp	Asn	Pro	Tyr	Leu	Lys	Tyr	Asn	His	Ala	
			85						90					95		
Asp	Ala	Glu	Phe	Gln	Glu	Arg	Leu	Lys	Glu	Asp	Thr	Ser	Phe	Gly	Gly	
			100					105					110			
Asn	Leu	Gly	Arg	Ala	Val	Phe	Gln	Ala	Lys	Lys	Arg	Val	Leu	Glu	Pro	
		115					120					125				
Leu	Gly	Leu	Val	Glu	Glu	Pro	Val	Lys	Thr	Ala	Pro	Gly	Lys	Lys	Arg	
	130					135					140					

Pro	Val	Glu	His	Ser	Pro	Val	Glu	Pro	Asp	Ser	Ser	Ser	Gly	Thr	Gly
145				150					155						160
Lys	Ala	Gly	Gln	Gln	Pro	Ala	Arg	Lys	Arg	Leu	Asn	Phe	Gly	Gln	Thr
			165					170						175	
Gly	Asp	Ala	Asp	Ser	Val	Pro	Asp	Pro	Gln	Pro	Leu	Gly	Gln	Pro	Pro
			180					185					190		
Ala	Ala	Pro	Ser	Gly	Leu	Gly	Thr	Asn	Thr	Met	Ala	Thr	Gly	Ser	Gly
		195					200					205			
Ala	Pro	Met	Ala	Asp	Asn	Asn	Glu	Gly	Ala	Asp	Gly	Val	Gly	Asn	Ser
	210				215					220					
Ser	Gly	Asn	Trp	His	Cys	Asp	Ser	Thr	Trp	Met	Gly	Asp	Arg	Val	Ile
225				230						235					240
Thr	Thr	Ser	Thr	Arg	Thr	Trp	Ala	Leu	Pro	Thr	Tyr	Asn	Asn	His	Leu
				245					250					255	
Tyr	Lys	Gln	Ile	Ser	Ser	Gln	Ser	Gly	Ala	Ser	Asn	Asp	Asn	His	Tyr
			260					265					270		
Phe	Gly	Tyr	Ser	Thr	Pro	Trp	Gly	Tyr	Phe	Asp	Phe	Asn	Arg	Phe	His
	275						280					285			
Cys	His	Phe	Ser	Pro	Arg	Asp	Trp	Gln	Arg	Leu	Ile	Asn	Asn	Asn	Trp
	290					295					300				
Gly	Phe	Arg	Pro	Lys	Arg	Leu	Asn	Phe	Lys	Leu	Phe	Asn	Ile	Gln	Val
305					310					315					320
Lys	Glu	Val	Thr	Gln	Asn	Asp	Gly	Thr	Thr	Thr	Ile	Ala	Asn	Asn	Leu
				325					330					335	
Thr	Ser	Thr	Val	Gln	Val	Phe	Thr	Asp	Ser	Glu	Tyr	Gln	Leu	Pro	Tyr
			340					345					350		
Val	Leu	Gly	Ser	Ala	His	Gln	Gly	Cys	Leu	Pro	Pro	Phe	Pro	Ala	Asp
		355					360					365			
Val	Phe	Met	Val	Pro	Gln	Tyr	Gly	Tyr	Leu	Thr	Leu	Asn	Asn	Gly	Ser
	370					375					380				
Gln	Ala	Val	Gly	Arg	Ser	Phe	Tyr	Cys	Leu	Glu	Tyr	Phe	Pro	Ser	
385					390				395					400	
Gln	Met	Leu	Arg	Thr	Gly	Asn	Asn	Phe	Thr	Phe	Ser	Tyr	Thr	Phe	Glu
				405					410					415	
Asp	Val	Pro	Phe	His	Ser	Ser	Tyr	Ala	His	Ser	Gln	Ser	Leu	Asp	Arg
			420					425					430		
Leu	Met	Asn	Pro	Leu	Ile	Asp	Gln	Tyr	Leu	Tyr	Tyr	Leu	Ser	Arg	Thr
		435				440						445			
Asn	Thr	Pro	Ser	Gly	Thr	Thr	Thr	Gln	Ser	Arg	Leu	Gln	Phe	Ser	Gln
	450					455					460				
Ala	Gly	Ala	Ser	Asp	Ile	Arg	Asp	Gln	Ser	Arg	Asn	Trp	Leu	Pro	Gly
465					470					475					480
Pro	Cys	Tyr	Arg	Gln	Gln	Arg	Val	Ser	Lys	Thr	Ser	Ala	Asp	Asn	Asn
				485					490					495	
Asn	Ser	Glu	Tyr	Ser	Trp	Thr	Gly	Ala	Thr	Lys	Tyr	His	Leu	Asn	Gly
			500					505					510		
Arg	Asp	Ser	Leu	Val	Asn	Pro	Gly	Pro	Ala	Met	Ala	Ser	His	Lys	Asp
		515					520					525			
Asp	Glu	Glu	Lys	Phe	Phe	Pro	Gln	Ser	Gly	Val	Leu	Ile	Phe	Gly	Lys
	530					535					540				
Gln	Gly	Ser	Glu	Lys	Thr	Asn	Val	Asp	Ile	Glu	Lys	Val	Met	Ile	Thr
545					550					555					560
Asp	Glu	Glu	Glu	Ile	Arg	Thr	Thr	Asn	Pro	Val	Ala	Thr	Glu	Gln	Tyr
				565					570					575	
Gly	Ser	Val	Ser	Thr	Asn	Leu	Gln	Arg	Gly	Asn	Arg	Gln	Ala	Ala	Thr
				580					585				590		
Ala	Asp	Val	Asn	Thr	Gln	Gly	Val	Leu	Pro	Gly	Met	Val	Trp	Gln	Asp
		595					600					605			
Arg	Asp	Val	Tyr	Leu	Gln	Gly	Pro	Ile	Trp	Ala	Lys	Ile	Pro	His	Thr
	610					615					620				
Asp	Gly	His	Phe	His	Pro	Ser	Pro	Leu	Met	Gly	Gly	Phe	Gly	Leu	Lys
625					630					635					640
His	Pro	Pro	Pro	Gln	Ile	Leu	Ile	Lys	Asn	Thr	Pro	Val	Pro	Ala	Asn
				645					650					655	
Pro	Ser	Thr	Thr	Phe	Ser	Ala	Ala	Lys	Phe	Ala	Ser	Phe	Ile	Thr	Gln
			660					665					670		
Tyr	Ser	Thr	Gly	Gln	Val	Ser	Val	Glu	Ile	Glu	Trp	Glu	Leu	Gln	Lys
		675					680					685			
Glu	Asn	Ser	Lys	Arg	Trp	Asn	Pro	Glu	Ile	Gln	Tyr	Thr	Ser	Asn	Tyr

690	695	700
Asn Lys Ser Val Asn Val Asp Phe Thr Val Asp Thr Asn Gly Val Tyr		
705	710	715
Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu		720
	725	730
		735

<210> 14
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 14
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 1 5

<210> 15
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 15
 Asn Glu Thr Ile Thr Arg Pro
 1 5

<210> 16
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 16
 Lys Ala Gly Gln Ala Asn Asn
 1 5

<210> 17
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 17
 Lys Asp Pro Lys Thr Thr Asn
 1 5

<210> 18
 <211> 7
 <212> PRT
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<220>
 <223> Synthetic Construct

<400> 18
 Lys Asp Thr Asp Thr Thr Arg

1 5

<210> 19
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<212> PRT
<213> Artificial Sequence

<220>
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<400> 19
Arg Ala Gly Gly Ser Val Gly
1 5

<210> 20
<211> 7
<212> PRT
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<220>
<223> Synthetic Construct

<400> 20
Ala Val Asp Thr Thr Lys Phe
1 5

<210> 21
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 21
Ser Thr Gly Lys Val Pro Asn
1 5

<210> 22
<211> 293
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 22
acttacggta aatggcccg cttggtgacc gcccaacgac ccccgcccat tgacgtcaat 60
aatgacgtat gttcccatag taacgccaat agggactttc cattgacgtc aatgggtgga 120
gtatttacgg taaactgccc acttggcagt acatcaagt tatcatatgc caagtccgcc 180
ccctattgac gtcaatgacg gtaaatggcc cgcctggcat tatgcccagt acatgacctt 240
acgggacttt cctacttggc agtacatcta cgtattagtc atcgctatta cca 293

<210> 23
<211> 220
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 23
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ttccaagtct ccacccatt gacgtcaatg ggagtttgtt ttggcaccaa aatcaacggg 120
actttccaaa atgtcgtaat aaccccgccc cgttgacgca aatgggcggt aggcgtgtac 180

ggtgggaggt ctatataagc agagctcgtt tagtgaaccg 220

<210> 24
<211> 318
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 24
ctcactctct tccgcatcgc tgtctgcgag ggccagctgt tgggctcgcg gttgaggaca 60
aactcttcgc ggtctttcca gtactcttgg atcggaacc cgtcggcctc cgaacggtac 120
tccgccaccg agggacctga gcgagtcgc atcgaccgga tcggaaaacc tctcgagaaa 180
ggcgtctaac cagtcacagt cgcaaggtag gctgagcacc gtggcgggcg gcagcgggtg 240
gcggtcgggg ttgtttctgg cggagggtgct gctgatgatg taattaaagt aggcggctct 300
gagacggcgg atggtcga 318

<210> 25
<211> 102
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 25
ccagctgttg gggtagtac tccctctcaa aagcgggcat tacttctgcg ctaagattgt 60
cagtttccaa aaacgaggag gatttgatat tcacctggcc cg 102

<210> 26
<211> 810
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 26
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gccttttaaat atttctctaa tttagtgtat atgcaatgat agttctctga tttctgagat 120
tgagtttctc atgtgtaatg attatttaga gtttctcttt catctgttca aatttttgtc 180
tagttttatt ttttactgat ttgtaagact tctttttata atctgcata tacaattctc 240
tttactgggg tgttgcaaat attttctgtc attctatggc ctgacttttc ttaatggttt 300
tttaatttta aaaataagtc ttaatatcca tgcaatctaa ttaacaatct tttctttgtg 360
gttaggactt tgagtcataa gaaatttttc tctacactga agtcatgatg gcatgcttct 420
atattatttt ctaaaagatt taaagttttg ccttctccat ttagacttat aattcactgg 480
aatttttttg tgtgtatggt atgacatatg gggtcccttt tattttttac atataaatat 540
atttccctgt ttttctaata aagaaaaaga tcatctttt cccattgtaa aatgccatat 600
ttttttcata ggtcacttac atatatcaat gggctctgtt ctgagctcta ctctatttta 660
tcagcctcac tgtctatccc cacacatctc atgctttgct ctaaatcttg atatttagtg 720
gaacattctt tcccattttg ttctacaaga atattttgt tattgtcttt gggctttcta 780
tatacathtt gaaatgaggt tgacaagtta 810

<210> 27
<211> 202
<212> DNA
<213> Artificial Sequence

<220>
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gtgtccttct ataataattat ggggtggagg ggggtggtat ggagcaaggg gcccaagttg 180
ggaagaaacc tgtaggcct gc 202

<210> 28

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

<220>
<223> Synthetic Construct

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tgggggtga 68

<210> 29
<211> 129
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

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atctcgttta gtgaaccgtc agatcctcac tctcttccgc atcgctgtct gcgagggcca 120
gctgttggg 129

<210> 30
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 30
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<210> 31
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 31
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<210> 32
<211> 77
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 32
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ttcctgcccc ggtggca 77

<210> 33
<211> 1172
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 33
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ttgacgtcaa taatgacgta tgttcccata gtaacgcaa tagggacttt ccattgacgt 120

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caatgggtgg agtattttac gtaaactgcc cacttggcag tacatcaagt gtatcatatg 180
ccaagtccgc cccctattga cgtcaatgac ggtaaatggc ccgcctggca ttatgcccag 240
tacatgacct tacgggactt tcctacttgg cagtacatct acgtattagt catcgctatt 300
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cgggactttc caaaatgtcg taataacccc gccccgttga cgcaaatggg cggtaggcgt 480
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tcaggggaga tctcgtttag tgaaccgtca gatcctcact ctcttccgca tcgctgtctg 660
cgagggccag ctgttgggct cgcgggttag gacaaactct tcgcgggtct tccagtactc 720
ttggatcgga aaccgcgtcg cctccgaacg gtactccgcc accgaggac ctgagcgagt 780
ccgcatcgac cggatcgga aacctctcga gaaaggcgtc taaccagtca cagtcgcaag 840
gtaggctgag caccgtggcg ggccgcagcg ggtggcggtc ggggttgttt ctggcggagg 900
tgctgctgat gatgtaatta aagtaggcgg tcttgagacg gcggatggtc gaggtgaggt 960
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1172

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<210> 34

<211> 1043

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 34

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ttgagtttct catgtgtaat gattattttag agtttctctt tcatctgttc aaatttttgt 180
ctagttttat tttttactga ttgtgaagac ttctttttat aatctgcata ttacaattct 240
ctttactggg gtgttgcaaa tattttctgt cattctatgg cctgactttt cttaatgggt 300
ttttaatttt aaaaaaaggt cttaatatct atgcaatcta attaacaatc ttttctttgt 360
ggttaggact ttgagtcata agaaattttt ctctacactg aagtcatgat ggcatgcttc 420
tatattattt tctaaaagat ttaaagtttt gccttctcca tttagactta taattcactg 480
gaattttttt gtgtgtatgg tatgacatat gggttccctt ttatttttta catataaata 540
tatttccctg tttttctaaa aaagaaaaag atcatcattt tccattgta aaatgccata 600
tttttttcat aggtcactta catatatcaa tgggtctgtt tctgagctct actctatttt 660
atcagcctca ctgtctatcc ccacacatct catgctttgc tctaaatctt gatatttagt 720
ggaacattct ttcccatttt gttctacaag aatatttttg ttattgtctt tgggctttct 780
atatacatat tgaatagagg ttgacaagtt acctaggaaa actgtcttcc tgcccgggtg 840
gcacccctgt gacccctccc cagtgcctct cctggccctg gaagttgcca ctccagtgcc 900
caccagcctt gtccataata aattaagttg catcattttg tctgactagg tgccttcta 960
taatattatg ggggtggagg gggtgggtat gagcaagggg cccaagttgg gaagaaacct 1020
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1043

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<210> 35

<211> 431

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 35

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Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
 1          5          10          15
Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
 20        25        30
Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
 35        40        45
Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
 50        55        60
Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
 65        70        75        80
Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
 85        90        95
Gln Thr Asn Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile

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100					105					110					
Glu	Leu	Ser	Val	Gly	Glu	Lys	Leu	Val	Leu	Asn	Cys	Thr	Ala	Arg	Thr
115					120					125					
Glu	Leu	Asn	Val	Gly	Ile	Asp	Phe	Asn	Trp	Glu	Tyr	Pro	Ser	Ser	Lys
130					135					140					
His	Gln	His	Lys	Lys	Leu	Val	Asn	Arg	Asp	Leu	Lys	Thr	Gln	Ser	Gly
145					150					155					
Ser	Glu	Met	Lys	Lys	Phe	Leu	Ser	Thr	Leu	Thr	Ile	Asp	Gly	Val	Thr
165					170					175					
Arg	Ser	Asp	Gln	Gly	Leu	Tyr	Thr	Cys	Ala	Ala	Ser	Ser	Gly	Leu	Met
180					185					190					
Thr	Lys	Lys	Asn	Ser	Thr	Phe	Val	Arg	Val	His	Glu	Lys	Asp	Lys	Thr
195					200					205					
His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser
210					215					220					
Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg
225					230					235					
Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro
245					250					255					
Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala
260					265					270					
Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val
275					280					285					
Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr
290					295					300					
Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr
305					310					315					
Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu
325					330					335					
Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys
340					345					350					
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser
355					360					365					
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp
370					375					380					
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser
385					390					395					
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala
405					410					415					
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	
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<210> 36

<211> 1377

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 36

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cccgaattaa tacacatgac tgaaggaagg gagctcgtca ttccctgccg ggttacgtca 180
cctaacaatca ctgttacttt aaaaaagttt ccacttgaca ctttgatccc tgatggaaaa 240
cgcataatct gggacagtag aaagggcttc atcatatcaa atgcaacgta caaagaaata 300
gggcttctga cctgtgaagc aacagtcaat gggcatttgt ataagacaaa ctatctcaca 360
catcgacaaa ccaataacaat catagatgtg gttctgagtc cgtctcatgg aattgaacta 420
tctgttgagg aaaagcttgt cttaaattgt acagcaagaa ctgaaactaa tgtggggatt 480
gacttcaact gggaataccc ttcttcgaag catcagcata agaaacttgt aaaccgagac 540
ctaaaaaacc agtctgggag tgagatgaag aaatttttga gcaccttaac tatagatggt 600
gtaacccgga gtgaccaagg attgtacacc tgtgcagcat ccagtgggct gatgaccaag 660
aagaacagca catttgtcag ggtcatgaa aaggacaaaa ctcacacatg cccaccgtgc 720
ccagcacctg aactcctggg gggaccgtca gtcttcctct tcccccaaaa acccaaggac 780
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gacctgagg tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca 900
aagccgcggg aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg 960
caccaggact ggctgaatgg caaggagtac aagtgcagg tctccaacaa agccctccca 1020

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gcccccatcg agaaaaccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac 1080
accctgcccc catcccggga tgagctgacc aagaaccagg tcagcctgac ctgcctgggtc 1140
aaaggcttct atcccagcga catcgccgtg gagtggggaga gcaatgggca gccggagAAC 1200
aactacaaga ccacgcctcc cgtgctggac tccgacggct ctttcttcct ctacagcaag 1260
ctcaccgtgg acaagagcag gtggcagcag gggaacgtct tctcatgctc cgtgatgcat 1320
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<210> 37

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 37

His Lys Phe Lys Ser Gly Asp

1 5

<210> 38

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<220>

<221> VARIANT

<222> 1, 2, 10

<223> Xaa = Any Amino Acid

<400> 38

Xaa Xaa His Lys Phe Lys Ser Gly Asp Xaa

1 5 10

<210> 39

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 39

Leu Ala His Lys Phe Lys Ser Gly Asp Ala

1 5 10

<210> 40

<211> 724

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 40

Met Ser Phe Val Asp His Pro Pro Asp Trp Leu Glu Glu Val Gly Glu

1 5 10 15

Gly Leu Arg Glu Phe Leu Gly Leu Glu Ala Gly Pro Pro Lys Pro Lys

20 25 30

Pro Asn Gln Gln His Gln Asp Gln Ala Arg Gly Leu Val Leu Pro Gly

35 40 45

Tyr Asn Tyr Leu Gly Pro Gly Asn Gly Leu Asp Arg Gly Glu Pro Val

50 55 60

Asn Arg Ala Asp Glu Val Ala Arg Glu His Asp Ile Ser Tyr Asn Glu

65											70											75											80
Gln	Leu	Glu	Ala	Gly	Asp	Asn	Pro	Tyr	Leu	Lys	Tyr	Asn	His	Ala	Asp																		
				85											90											95							
Ala	Glu	Phe	Gln	Glu	Lys	Leu	Ala	Asp	Asp	Thr	Ser	Phe	Gly	Gly	Asn																		
				100											105											110							
Leu	Gly	Lys	Ala	Val	Phe	Gln	Ala	Lys	Lys	Arg	Val	Leu	Glu	Pro	Phe																		
				115											120											125							
Gly	Leu	Val	Glu	Glu	Gly	Ala	Lys	Thr	Ala	Pro	Thr	Gly	Lys	Arg	Ile																		
				130											135											140							
Asp	Asp	His	Phe	Pro	Lys	Arg	Lys	Lys	Ala	Arg	Thr	Glu	Glu	Asp	Ser																		
				145											150											155							
Lys	Pro	Ser	Thr	Ser	Ser	Asp	Ala	Glu	Ala	Gly	Pro	Ser	Gly	Ser	Gln																		
				165											170											175							
Gln	Leu	Gln	Ile	Pro	Ala	Gln	Pro	Ala	Ser	Ser	Leu	Gly	Ala	Asp	Thr																		
				180											185											190							
Met	Ser	Ala	Gly	Gly	Gly	Gly	Pro	Leu	Gly	Asp	Asn	Asn	Gln	Gly	Ala																		
				195											200											205							
Asp	Gly	Val	Gly	Asn	Ala	Ser	Gly	Asp	Trp	His	Cys	Asp	Ser	Thr	Trp																		
				210											215											220							
Met	Gly	Asp	Arg	Val	Val	Thr	Lys	Ser	Thr	Arg	Thr	Trp	Val	Leu	Pro																		
				225											230											235							
Ser	Tyr	Asn	Asn	His	Gln	Tyr	Arg	Glu	Ile	Lys	Ser	Gly	Ser	Val	Asp																		
				245											250											255							
Gly	Ser	Asn	Ala	Asn	Ala	Tyr	Phe	Gly	Tyr	Ser	Thr	Pro	Trp	Gly	Tyr																		
				260											265											270							
Phe	Asp	Phe	Asn	Arg	Phe	His	Ser	His	Trp	Ser	Pro	Arg	Asp	Trp	Gln																		
				275											280											285							
Arg	Leu	Ile	Asn	Asn	Tyr	Trp	Gly	Phe	Arg	Pro	Arg	Ser	Leu	Arg	Val																		
				290											295											300							
Lys	Ile	Phe	Asn	Ile	Gln	Val	Lys	Glu	Val	Thr	Val	Gln	Asp	Ser	Thr																		
				305											310											315							
Thr	Thr	Ile	Ala	Asn	Asn	Leu	Thr	Ser	Thr	Val	Gln	Val	Phe	Thr	Asp																		
				325											330											335							
Asp	Asp	Tyr	Gln	Leu	Pro	Tyr	Val	Val	Gly	Asn	Gly	Thr	Glu	Gly	Cys																		
				340											345											350							
Leu	Pro	Ala	Phe	Pro	Pro	Gln	Val	Phe	Thr	Leu	Pro	Gln	Tyr	Gly	Tyr																		
				355											360											365							
Ala	Thr	Leu	Asn	Arg	Asp	Asn	Thr	Glu	Asn	Pro	Thr	Glu	Arg	Ser	Ser																		
				370											375											380							
Phe	Phe	Cys	Leu	Glu	Tyr	Phe	Pro	Ser	Lys	Met	Leu	Arg	Thr	Gly	Asn																		
				385											390											395							
Asn	Phe	Glu	Phe	Thr	Tyr	Asn	Phe	Glu	Glu	Val	Pro	Phe	His	Ser	Ser																		
				405											410											415							
Phe	Ala	Pro	Ser	Gln	Asn	Leu	Phe	Lys	Leu	Ala	Asn	Pro	Leu	Val	Asp																		
				420											425											430							
Gln	Tyr	Leu	Tyr	Arg	Phe	Val	Ser	Thr	Asn	Asn	Thr	Gly	Gly	Val	Gln																		
				435											440											445							
Phe	Asn	Lys	Asn	Leu	Ala	Gly	Arg	Tyr	Ala	Asn	Thr	Tyr	Lys	Asn	Trp																		
				450											455											460							
Phe	Pro	Gly	Pro	Met	Gly	Arg	Thr	Gln	Gly	Trp	Asn	Leu	Gly	Ser	Gly																		
				465											470											475							
Val	Asn	Arg	Ala	Ser	Val	Ser	Ala	Phe	Ala	Thr	Thr	Asn	Arg	Met	Glu																		
				485											490											495							
Leu	Glu	Gly	Ala	Ser	Tyr	Gln	Val	Pro	Pro	Gln	Pro	Asn	Gly	Met	Thr																		
				500											505											510							
Asn	Asn	Leu	Gln	Gly	Ser	Asn	Thr	Tyr	Ala	Leu	Glu	Asn	Thr	Met	Ile																		
				515											520											525							
Phe	Asn	Ser	Gln	Pro	Ala	Asn	Pro	Gly	Thr	Thr	Ala	Thr	Tyr	Leu	Glu																		
				530											535											540							
Gly	Asn	Met	Leu	Ile	Thr	Ser	Glu	Ser	Glu	Thr	Gln	Pro	Val	Asn	Arg																		
				545											550											555							
Val	Ala	Tyr	Asn	Val	Gly	Gly	Gln	Met	Ala	Thr	Asn	Asn	Gln	Ser	Ser																		
				565											570											575							
Thr	Thr	Ala	Pro	Ala	Thr	Gly	Thr	Tyr	Asn	Leu	Gln	Glu	Ile	Val	Pro																		
				580											585											590							
Gly	Ser	Val	Trp	Met	Glu	Arg	Asp	Val	Tyr	Leu	Gln	Gly	Pro	Ile	Trp																		
				595											600											605							
Ala	Lys	Ile	Pro	Glu	Thr	Gly	Ala	His	Phe	His	Pro	Ser	Pro	Ala	Met																		
				610											615											620							

Gly 625	Gly	Phe	Gly	Leu	Lys 630	His	Pro	Pro	Pro	Met 635	Met	Leu	Ile	Lys	Asn 640
Thr	Pro	Val	Pro	Gly 645	Asn	Ile	Thr	Ser	Phe	Ser 650	Asp	Val	Pro	Val	Ser
Ser	Phe	Ile	Thr	Gln	Tyr	Ser	Thr	Gly 665	Gln	Val	Thr	Val	Glu	Met	Glu
Trp	Glu	Leu	Lys 675	Lys	Glu	Asn	Ser 680	Lys	Arg	Trp	Asn	Pro	Glu	Ile	Gln
Tyr	Thr	Asn	Asn	Tyr	Asn	Asp	Pro	Gln	Phe	Val	Asp	Phe	Ala	Pro	Asp
Ser 705	Thr	Gly	Glu	Tyr	Arg	Thr	Thr	Arg	Pro	Ile	Gly	Thr	Arg	Tyr	Leu
Thr	Arg	Pro	Leu												

<210> 41
 <211> 725
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 41

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Glu	Gly	Ile	Arg	Gln	Trp	Trp	Lys	Leu	Lys	Pro	Gly	Pro	Pro	Pro	Pro
Lys	Pro	Ala	Glu	Arg	His	Lys	Asp	Asp	Ser	Arg	Gly	Leu	Val	Leu	Pro
Gly	Tyr	Lys	Tyr	Leu	Gly	Pro	Phe	Asn	Gly	Leu	Asp	Lys	Gly	Glu	Pro
Val	Asn	Glu	Ala	Asp	Ala	Ala	Leu	Glu	His	Asp	Lys	Ala	Tyr	Asp	
Arg	Gln	Leu	Asp	Ser	Gly	Asp	Asn	Pro	Tyr	Leu	Lys	Tyr	Asn	His	Ala
Asp	Ala	Glu	Phe	Gln	Glu	Arg	Leu	Lys	Glu	Asp	Thr	Ser	Phe	Gly	Gly
Asn	Leu	Gly	Arg	Ala	Val	Phe	Gln	Ala	Lys	Lys	Arg	Val	Leu	Glu	Pro
Phe	Gly	Leu	Val	Glu	Glu	Gly	Ala	Lys	Thr	Ala	Pro	Thr	Gly	Lys	Arg
Ile	Asp	Asp	His	Phe	Pro	Lys	Arg	Lys	Lys	Ala	Arg	Thr	Glu	Glu	Asp
Ser	Lys	Pro	Ser	Thr	Ser	Ser	Asp	Ala	Glu	Ala	Gly	Pro	Ser	Gly	Ser
Gln	Gln	Leu	Gln	Ile	Pro	Ala	Gln	Pro	Ala	Ser	Ser	Leu	Gly	Ala	Asp
Thr	Met	Ser	Ala	Gly	Gly	Gly	Gly	Pro	Leu	Gly	Asp	Asn	Asn	Gln	Gly
Ala	Asp	Gly	Val	Gly	Asn	Ala	Ser	Gly	Asp	Trp	His	Cys	Asp	Ser	Thr
Trp	Met	Gly	Asp	Arg	Val	Val	Thr	Lys	Ser	Thr	Arg	Thr	Trp	Val	Leu
Pro	Ser	Tyr	Asn	Asn	His	Gln	Tyr	Arg	Glu	Ile	Lys	Ser	Gly	Ser	Val
Asp	Gly	Ser	Asn	Ala	Asn	Ala	Tyr	Phe	Gly	Tyr	Ser	Thr	Pro	Trp	Gly
Tyr	Phe	Asp	Phe	Asn	Arg	Phe	His	Ser	His	Trp	Ser	Pro	Arg	Asp	Trp
Gln	Arg	Leu	Ile	Asn	Asn	Tyr	Trp	Gly	Phe	Arg	Pro	Arg	Ser	Leu	Arg
Val	Lys	Ile	Phe	Asn	Ile	Gln	Val	Lys	Glu	Val	Thr	Val	Gln	Asp	Ser
Thr	Thr	Thr	Ile	Ala	Asn	Asn	Leu	Thr	Ser	Thr	Val	Gln	Val	Phe	Thr
Asp	Asp	Asp	Tyr	Gln	Leu	Pro	Tyr	Val	Val	Gly	Asn	Gly	Thr	Glu	Gly

Cys	Leu	Pro	Ala	Phe	Pro	Pro	Gln	Val	Phe	Thr	Leu	Pro	Gln	Tyr	Gly
		355					360					365			
Tyr	Ala	Thr	Leu	Asn	Arg	Asp	Asn	Thr	Glu	Asn	Pro	Thr	Glu	Arg	Ser
	370					375					380				
Ser	Phe	Phe	Cys	Leu	Glu	Tyr	Phe	Pro	Ser	Lys	Met	Leu	Arg	Thr	Gly
385					390					395					400
Asn	Asn	Phe	Glu	Phe	Thr	Tyr	Asn	Phe	Glu	Glu	Val	Pro	Phe	His	Ser
			405						410					415	
Ser	Phe	Ala	Pro	Ser	Gln	Asn	Leu	Phe	Lys	Leu	Ala	Asn	Pro	Leu	Val
			420						425				430		
Asp	Gln	Tyr	Leu	Tyr	Arg	Phe	Val	Ser	Thr	Asn	Asn	Thr	Gly	Gly	Val
		435					440					445			
Gln	Phe	Asn	Lys	Asn	Leu	Ala	Gly	Arg	Tyr	Ala	Asn	Thr	Tyr	Lys	Asn
	450					455					460				
Trp	Phe	Pro	Gly	Pro	Met	Gly	Arg	Thr	Gln	Gly	Trp	Asn	Leu	Gly	Ser
465					470					475					480
Gly	Val	Asn	Arg	Ala	Ser	Val	Ser	Ala	Phe	Ala	Thr	Thr	Asn	Arg	Met
			485						490					495	
Glu	Leu	Glu	Gly	Ala	Ser	Tyr	Gln	Val	Pro	Pro	Gln	Pro	Asn	Gly	Met
			500					505					510		
Thr	Asn	Asn	Leu	Gln	Gly	Ser	Asn	Thr	Tyr	Ala	Leu	Glu	Asn	Thr	Met
		515					520					525			
Ile	Phe	Asn	Ser	Gln	Pro	Ala	Asn	Pro	Gly	Thr	Thr	Ala	Thr	Tyr	Leu
	530					535					540				
Glu	Gly	Asn	Met	Leu	Ile	Thr	Ser	Glu	Ser	Glu	Thr	Gln	Pro	Val	Asn
545					550					555					560
Arg	Val	Ala	Tyr	Asn	Val	Gly	Gly	Gln	Met	Ala	Thr	Asn	Asn	Gln	Ser
			565						570					575	
Ser	Thr	Thr	Ala	Pro	Thr	Thr	Gly	Thr	Tyr	Asn	Leu	Gln	Glu	Ile	Val
			580					585					590		
Pro	Gly	Ser	Val	Trp	Met	Glu	Arg	Asp	Val	Tyr	Leu	Gln	Gly	Pro	Ile
		595				600						605			
Trp	Ala	Lys	Ile	Pro	Glu	Thr	Gly	Ala	His	Phe	His	Pro	Ser	Pro	Ala
	610					615					620				
Met	Gly	Gly	Phe	Gly	Leu	Lys	His	Pro	Pro	Pro	Met	Met	Leu	Ile	Lys
625					630					635					640
Asn	Thr	Pro	Val	Pro	Gly	Asn	Ile	Thr	Ser	Phe	Ser	Asp	Val	Pro	Val
			645						650					655	
Ser	Ser	Phe	Ile	Thr	Gln	Tyr	Ser	Thr	Gly	Gln	Val	Thr	Val	Glu	Met
			660						665				670		
Glu	Trp	Glu	Leu	Lys	Lys	Glu	Asn	Ser	Lys	Arg	Trp	Asn	Pro	Glu	Ile
		675					680					685			
Gln	Tyr	Thr	Asn	Asn	Tyr	Asn	Asp	Pro	Gln	Phe	Val	Asp	Phe	Ala	Pro
	690					695					700				
Asp	Ser	Thr	Gly	Glu	Tyr	Arg	Thr	Thr	Arg	Pro	Ile	Gly	Thr	Arg	Tyr
705					710					715					720
Leu	Thr	Arg	Pro	Leu											
				725											

<210> 42
 <211> 724
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 42
 Met Ser Phe Val Asp His Pro Pro Asp Trp Leu Glu Glu Val Gly Glu
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 Gly Leu Arg Glu Phe Leu Gly Leu Glu Ala Gly Pro Pro Lys Pro Lys
 20 25 30
 Pro Asn Gln His Gln Asp Gln Ala Arg Gly Leu Val Leu Pro Gly
 35 40 45
 Tyr Asn Tyr Leu Gly Pro Gly Asn Gly Leu Asp Arg Gly Glu Pro Val
 50 55 60
 Asn Arg Ala Asp Glu Val Ala Arg Glu His Asp Ile Ser Tyr Asn Glu
 65 70 75 80

Gln	Leu	Glu	Ala	Gly	Asp	Asn	Pro	Tyr	Leu	Lys	Tyr	Asn	His	Ala	Asp	85	90	95
Ala	Glu	Phe	Gln	Glu	Lys	Leu	Ala	Asp	Asp	Thr	Ser	Phe	Gly	Gly	Asn	100	105	110
Leu	Gly	Lys	Ala	Val	Phe	Gln	Ala	Lys	Lys	Arg	Val	Leu	Glu	Pro	Phe	115	120	125
Gly	Leu	Val	Glu	Glu	Gly	Ala	Lys	Thr	Ala	Pro	Thr	Gly	Lys	Arg	Ile	130	135	140
Asp	Asp	His	Phe	Pro	Lys	Arg	Lys	Lys	Ala	Arg	Thr	Glu	Glu	Asp	Ser	145	150	155
Lys	Pro	Ser	Thr	Ser	Ser	Asp	Ala	Glu	Ala	Gly	Pro	Ser	Gly	Ser	Gln	165	170	175
Gln	Leu	Gln	Ile	Pro	Ala	Gln	Pro	Ala	Ser	Ser	Leu	Gly	Ala	Asp	Thr	180	185	190
Met	Ser	Ala	Gly	Gly	Gly	Gly	Pro	Leu	Gly	Asp	Asn	Asn	Gln	Gly	Ala	195	200	205
Asp	Gly	Val	Gly	Asn	Ala	Ser	Gly	Asp	Trp	His	Cys	Asp	Ser	Thr	Trp	210	215	220
Met	Gly	Asp	Arg	Val	Val	Thr	Lys	Ser	Thr	Arg	Thr	Trp	Val	Leu	Pro	225	230	235
Ser	Tyr	Asn	Asn	His	Gln	Tyr	Arg	Glu	Ile	Lys	Ser	Gly	Ser	Val	Asp	245	250	255
Gly	Ser	Asn	Ala	Asn	Ala	Tyr	Phe	Gly	Tyr	Ser	Thr	Pro	Trp	Gly	Tyr	260	265	270
Phe	Asp	Phe	Asn	Arg	Phe	His	Ser	His	Trp	Ser	Pro	Arg	Asp	Trp	Gln	275	280	285
Arg	Leu	Ile	Asn	Asn	Tyr	Trp	Gly	Phe	Arg	Pro	Arg	Ser	Leu	Arg	Val	290	295	300
Lys	Ile	Phe	Asn	Ile	Gln	Val	Lys	Glu	Val	Thr	Val	Gln	Asp	Ser	Thr	305	310	315
Thr	Thr	Ile	Ala	Asn	Asn	Leu	Thr	Ser	Thr	Val	Gln	Val	Phe	Thr	Asp	325	330	335
Asp	Asp	Tyr	Gln	Leu	Pro	Tyr	Val	Val	Gly	Asn	Gly	Thr	Glu	Gly	Cys	340	345	350
Leu	Pro	Ala	Phe	Pro	Pro	Gln	Val	Phe	Thr	Leu	Pro	Gln	Tyr	Gly	Tyr	355	360	365
Ala	Thr	Leu	Asn	Arg	Asp	Asn	Thr	Glu	Asn	Pro	Thr	Glu	Arg	Ser	Ser	370	375	380
Phe	Phe	Cys	Leu	Glu	Tyr	Phe	Pro	Ser	Lys	Met	Leu	Arg	Thr	Gly	Asn	385	390	395
Asn	Phe	Glu	Phe	Thr	Tyr	Asn	Phe	Glu	Glu	Val	Pro	Phe	His	Ser	Ser	405	410	415
Phe	Ala	Pro	Ser	Gln	Asn	Leu	Phe	Lys	Leu	Ala	Asn	Pro	Leu	Val	Asp	420	425	430
Gln	Tyr	Leu	Tyr	Arg	Phe	Val	Ser	Thr	Asn	Asn	Thr	Gly	Gly	Val	Gln	435	440	445
Phe	Asn	Lys	Asn	Leu	Ala	Gly	Arg	Tyr	Ala	Asn	Thr	Tyr	Lys	Asn	Trp	450	455	460
Phe	Pro	Gly	Pro	Met	Gly	Arg	Thr	Gln	Gly	Trp	Asn	Leu	Gly	Ser	Gly	465	470	475
Val	Asn	Arg	Ala	Ser	Val	Ser	Ala	Phe	Ala	Thr	Thr	Asn	Arg	Met	Glu	485	490	495
Leu	Glu	Gly	Ala	Ser	Tyr	Gln	Val	Pro	Pro	Gln	Pro	Asn	Gly	Met	Thr	500	505	510
Asn	Asn	Leu	Gln	Gly	Ser	Asn	Thr	Tyr	Ala	Leu	Glu	Asn	Thr	Met	Ile	515	520	525
Phe	Asn	Ser	Gln	Pro	Ala	Asn	Pro	Gly	Thr	Thr	Ala	Thr	Tyr	Leu	Glu	530	535	540
Gly	Asn	Met	Leu	Ile	Thr	Ser	Glu	Ser	Glu	Thr	Gln	Pro	Val	Asn	Arg	545	550	555
Val	Ala	Tyr	Asn	Val	Gly	Gly	Gln	Met	Leu	Ala	His	Lys	Phe	Lys	Ser	565	570	575
Gly	Asp	Ala	Pro	Ala	Thr	Gly	Thr	Tyr	Asn	Leu	Gln	Glu	Ile	Val	Pro	580	585	590
Gly	Ser	Val	Trp	Met	Glu	Arg	Asp	Val	Tyr	Leu	Gln	Gly	Pro	Ile	Trp	595	600	605
Ala	Lys	Ile	Pro	Glu	Thr	Gly	Ala	His	Phe	His	Pro	Ser	Pro	Ala	Met	610	615	620
Gly	Gly	Phe	Gly	Leu	Lys	His	Pro	Pro	Pro	Met	Met	Leu	Ile	Lys	Asn			

625					630					635				640	
Thr	Pro	Val	Pro	Gly	Asn	Ile	Thr	Ser	Phe	Ser	Asp	Val	Pro	Val	Ser
				645					650					655	
Ser	Phe	Ile	Thr	Gln	Tyr	Ser	Thr	Gly	Gln	Val	Thr	Val	Glu	Met	Glu
			660					665					670		
Trp	Glu	Leu	Lys	Lys	Glu	Asn	Ser	Lys	Arg	Trp	Asn	Pro	Glu	Ile	Gln
		675					680				685				
Tyr	Thr	Asn	Asn	Tyr	Asn	Asp	Pro	Gln	Phe	Val	Asp	Phe	Ala	Pro	Asp
	690					695				700					
Ser	Thr	Gly	Glu	Tyr	Arg	Thr	Thr	Arg	Pro	Ile	Gly	Thr	Arg	Tyr	Leu
705					710					715					720
Thr	Arg	Pro	Leu												

<210> 43
 <211> 725
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic Construct

<400> 43

Met	Ala	Ala	Asp	Gly	Tyr	Leu	Pro	Asp	Trp	Leu	Glu	Asp	Thr	Leu	Ser
1				5				10					15		
Glu	Gly	Ile	Arg	Gln	Trp	Trp	Lys	Leu	Lys	Pro	Gly	Pro	Pro	Pro	Pro
			20				25					30			
Lys	Pro	Ala	Glu	Arg	His	Lys	Asp	Asp	Ser	Arg	Gly	Leu	Val	Leu	Pro
		35				40					45				
Gly	Tyr	Lys	Tyr	Leu	Gly	Pro	Phe	Asn	Gly	Leu	Asp	Lys	Gly	Glu	Pro
	50				55					60					
Val	Asn	Glu	Ala	Asp	Ala	Ala	Leu	Glu	His	Asp	Lys	Ala	Tyr	Asp	
65				70				75						80	
Arg	Gln	Leu	Asp	Ser	Gly	Asp	Asn	Pro	Tyr	Leu	Lys	Tyr	Asn	His	Ala
			85				90						95		
Asp	Ala	Glu	Phe	Gln	Glu	Arg	Leu	Lys	Glu	Asp	Thr	Ser	Phe	Gly	Gly
			100				105					110			
Asn	Leu	Gly	Arg	Ala	Val	Phe	Gln	Ala	Lys	Lys	Arg	Val	Leu	Glu	Pro
	115					120					125				
Phe	Gly	Leu	Val	Glu	Glu	Gly	Ala	Lys	Thr	Ala	Pro	Thr	Gly	Lys	Arg
	130					135				140					
Ile	Asp	Asp	His	Phe	Pro	Lys	Arg	Lys	Lys	Ala	Arg	Thr	Glu	Glu	Asp
145				150				155						160	
Ser	Lys	Pro	Ser	Thr	Ser	Ser	Asp	Ala	Glu	Ala	Gly	Pro	Ser	Gly	Ser
			165					170					175		
Gln	Gln	Leu	Gln	Ile	Pro	Ala	Gln	Pro	Ala	Ser	Ser	Leu	Gly	Ala	Asp
			180				185						190		
Thr	Met	Ser	Ala	Gly	Gly	Gly	Gly	Pro	Leu	Gly	Asp	Asn	Asn	Gln	Gly
	195					200						205			
Ala	Asp	Gly	Val	Gly	Asn	Ala	Ser	Gly	Asp	Trp	His	Cys	Asp	Ser	Thr
	210				215					220					
Trp	Met	Gly	Asp	Arg	Val	Val	Thr	Lys	Ser	Thr	Arg	Thr	Trp	Val	Leu
225				230				235						240	
Pro	Ser	Tyr	Asn	Asn	His	Gln	Tyr	Arg	Glu	Ile	Lys	Ser	Gly	Ser	Val
			245					250					255		
Asp	Gly	Ser	Asn	Ala	Asn	Ala	Tyr	Phe	Gly	Tyr	Ser	Thr	Pro	Trp	Gly
			260				265						270		
Tyr	Phe	Asp	Phe	Asn	Arg	Phe	His	Ser	His	Trp	Ser	Pro	Arg	Asp	Trp
	275					280						285			
Gln	Arg	Leu	Ile	Asn	Asn	Tyr	Trp	Gly	Phe	Arg	Pro	Arg	Ser	Leu	Arg
	290				295					300					
Val	Lys	Ile	Phe	Asn	Ile	Gln	Val	Lys	Glu	Val	Thr	Val	Gln	Asp	Ser
305				310				315						320	
Thr	Thr	Thr	Ile	Ala	Asn	Asn	Leu	Thr	Ser	Thr	Val	Gln	Val	Phe	Thr
			325					330					335		
Asp	Asp	Asp	Tyr	Gln	Leu	Pro	Tyr	Val	Val	Gly	Asn	Gly	Thr	Glu	Gly
			340				345					350			
Cys	Leu	Pro	Ala	Phe	Pro	Pro	Gln	Val	Phe	Thr	Leu	Pro	Gln	Tyr	Gly

355	360	365
Tyr Ala Thr Leu Asn Arg Asp Asn Thr Glu Asn Pro Thr Glu Arg Ser		
370	375	380
Ser Phe Phe Cys Leu Glu Tyr Phe Pro Ser Lys Met Leu Arg Thr Gly		
385	390	395
Asn Asn Phe Glu Phe Thr Tyr Asn Phe Glu Glu Val Pro Phe His Ser		
405	410	415
Ser Phe Ala Pro Ser Gln Asn Leu Phe Lys Leu Ala Asn Pro Leu Val		
420	425	430
Asp Gln Tyr Leu Tyr Arg Phe Val Ser Thr Asn Asn Thr Gly Gly Val		
435	440	445
Gln Phe Asn Lys Asn Leu Ala Gly Arg Tyr Ala Asn Thr Tyr Lys Asn		
450	455	460
Trp Phe Pro Gly Pro Met Gly Arg Thr Gln Gly Trp Asn Leu Gly Ser		
465	470	475
Gly Val Asn Arg Ala Ser Val Ser Ala Phe Ala Thr Thr Asn Arg Met		
485	490	495
Glu Leu Glu Gly Ala Ser Tyr Gln Val Pro Pro Gln Pro Asn Gly Met		
500	505	510
Thr Asn Asn Leu Gln Gly Ser Asn Thr Tyr Ala Leu Glu Asn Thr Met		
515	520	525
Ile Phe Asn Ser Gln Pro Ala Asn Pro Gly Thr Thr Ala Thr Tyr Leu		
530	535	540
Glu Gly Asn Met Leu Ile Thr Ser Glu Ser Glu Thr Gln Pro Val Asn		
545	550	555
Arg Val Ala Tyr Asn Val Gly Gly Gln Met Leu Ala His Lys Phe Lys		
565	570	575
Ser Gly Asp Ala Pro Thr Thr Gly Thr Tyr Asn Leu Gln Glu Ile Val		
580	585	590
Pro Gly Ser Val Trp Met Glu Arg Asp Val Tyr Leu Gln Gly Pro Ile		
595	600	605
Trp Ala Lys Ile Pro Glu Thr Gly Ala His Phe His Pro Ser Pro Ala		
610	615	620
Met Gly Gly Phe Gly Leu Lys His Pro Pro Pro Met Met Leu Ile Lys		
625	630	635
Asn Thr Pro Val Pro Gly Asn Ile Thr Ser Phe Ser Asp Val Pro Val		
645	650	655
Ser Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Thr Val Glu Met		
660	665	670
Glu Trp Glu Leu Lys Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile		
675	680	685
Gln Tyr Thr Asn Asn Tyr Asn Asp Pro Gln Phe Val Asp Phe Ala Pro		
690	695	700
Asp Ser Thr Gly Glu Tyr Arg Thr Thr Arg Pro Ile Gly Thr Arg Tyr		
705	710	715
Leu Thr Arg Pro Leu		
725		

<210> 44

<211> 587

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic Construct

<400> 44

Ala Pro Thr Gly Lys Arg Ile Asp Asp His Phe Pro Lys Arg Lys Lys		
1	5	10
Ala Arg Thr Glu Glu Asp Ser Lys Pro Ser Thr Ser Ser Asp Ala Glu		
20	25	30
Ala Gly Pro Ser Gly Ser Gln Gln Leu Gln Ile Pro Ala Gln Pro Ala		
35	40	45
Ser Ser Leu Gly Ala Asp Thr Met Ser Ala Gly Gly Gly Gly Pro Leu		
50	55	60
Gly Asp Asn Asn Gln Gly Ala Asp Gly Val Gly Asn Ala Ser Gly Asp		
65	70	75
Trp His Cys Asp Ser Thr Trp Met Gly Asp Arg Val Val Thr Lys Ser		

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<210> 45
<211> 532
<212> PRT
<213> Artificial Sequence
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<220>

<223> Synthetic Construct

<400> 45

Met	Ser	Ala	Gly	Gly	Gly	Gly	Pro	Leu	Gly	Asp	Asn	Asn	Gln	Gly	Ala	
1				5					10				15			
Asp	Gly	Val	Gly	Asn	Ala	Ser	Gly	Asp	Trp	His	Cys	Asp	Ser	Thr	Trp	
			20					25					30			
Met	Gly	Asp	Arg	Val	Val	Thr	Lys	Ser	Thr	Arg	Thr	Trp	Val	Leu	Pro	
		35					40					45				
Ser	Tyr	Asn	Asn	His	Gln	Tyr	Arg	Glu	Ile	Lys	Ser	Gly	Ser	Val	Asp	
	50					55					60					
Gly	Ser	Asn	Ala	Asn	Ala	Tyr	Phe	Gly	Tyr	Ser	Thr	Pro	Trp	Gly	Tyr	
65					70					75					80	
Phe	Asp	Phe	Asn	Arg	Phe	His	Ser	His	Trp	Ser	Pro	Arg	Asp	Trp	Gln	
				85					90					95		
Arg	Leu	Ile	Asn	Asn	Tyr	Trp	Gly	Phe	Arg	Pro	Arg	Ser	Leu	Arg	Val	
			100					105					110			
Lys	Ile	Phe	Asn	Ile	Gln	Val	Lys	Glu	Val	Thr	Val	Gln	Asp	Ser	Thr	
		115					120					125				
Thr	Thr	Ile	Ala	Asn	Asn	Leu	Thr	Ser	Thr	Val	Gln	Val	Phe	Thr	Asp	
	130					135					140					
Asp	Asp	Tyr	Gln	Leu	Pro	Tyr	Val	Val	Gly	Asn	Gly	Thr	Glu	Gly	Cys	
145					150					155					160	
Leu	Pro	Ala	Phe	Pro	Pro	Gln	Val	Phe	Thr	Leu	Pro	Gln	Tyr	Gly	Tyr	
				165					170					175		
Ala	Thr	Leu	Asn	Arg	Asp	Asn	Thr	Glu	Asn	Pro	Thr	Glu	Arg	Ser	Ser	
			180					185					190			
Phe	Phe	Cys	Leu	Glu	Tyr	Phe	Pro	Ser	Lys	Met	Leu	Arg	Thr	Gly	Asn	
		195					200					205				
Asn	Phe	Glu	Phe	Thr	Tyr	Asn	Phe	Glu	Glu	Val	Pro	Phe	His	Ser	Ser	
	210					215					220					
Phe	Ala	Pro	Ser	Gln	Asn	Leu	Phe	Lys	Leu	Ala	Asn	Pro	Leu	Val	Asp	
225					230					235					240	
Gln	Tyr	Leu	Tyr	Arg	Phe	Val	Ser	Thr	Asn	Asn	Thr	Gly	Gly	Val	Gln	
				245					250					255		
Phe	Asn	Lys	Asn	Leu	Ala	Gly	Arg	Tyr	Ala	Asn	Thr	Tyr	Lys	Asn	Trp	
			260					265					270			
Phe	Pro	Gly	Pro	Met	Gly	Arg	Thr	Gln	Gly	Trp	Asn	Leu	Gly	Ser	Gly	
		275					280					285				
Val	Asn	Arg	Ala	Ser	Val	Ser	Ala	Phe	Ala	Thr	Thr	Asn	Arg	Met	Glu	
	290					295					300					
Leu	Glu	Gly	Ala	Ser	Tyr	Gln	Val	Pro	Pro	Gln	Pro	Asn	Gly	Met	Thr	
305					310					315					320	
Asn	Asn	Leu	Gln	Gly	Ser	Asn	Thr	Tyr	Ala	Leu	Glu	Asn	Thr	Met	Ile	
				325					330					335		
Phe	Asn	Ser	Gln	Pro	Ala	Asn	Pro	Gly	Thr	Thr	Ala	Thr	Tyr	Leu	Glu	
			340					345					350			
Gly	Asn	Met	Leu	Ile	Thr	Ser	Glu	Ser	Glu	Thr	Gln	Pro	Val	Asn	Arg	
		355					360					365				
Val	Ala	Tyr	Asn	Val	Gly	Gly	Gln	Met	Leu	Ala	His	Lys	Phe	Lys	Ser	
	370					375					380					
Gly	Asp	Ala	Pro	Thr	Thr	Gly	Thr	Tyr	Asn	Leu	Gln	Glu	Ile	Val	Pro	
385					390					395					400	
Gly	Ser	Val	Trp	Met	Glu	Arg	Asp	Val	Tyr	Leu	Gln	Gly	Pro	Ile	Trp	
				405					410					415		
Ala	Lys	Ile	Pro	Glu	Thr	Gly	Ala	His	Phe	His	Pro	Ser	Pro	Ala	Met	
			420					425					430			
Gly	Gly	Phe	Gly	Leu	Lys	His	Pro	Pro	Pro	Met	Met	Leu	Ile	Lys	Asn	
		435					440					445				
Thr	Pro	Val	Pro	Gly	Asn	Ile	Thr	Ser	Phe	Ser	Asp	Val	Pro	Val	Ser	
	450					455					460					
Ser	Phe	Ile	Thr	Gln	Tyr	Ser	Thr	Gly	Gln	Val	Thr	Val	Glu	Met	Glu	
465					470					475					480	
Trp	Glu	Leu	Lys	Lys	Glu	Asn	Ser	Lys	Arg	Trp	Asn	Pro	Glu	Ile	Gln	
				485					490					495		
Tyr	Thr	Asn	Asn	Tyr	Asn	Asp	Pro	Gln	Phe	Val	Asp	Phe	Ala	Pro	Asp	
			500					505					510			

Ser Thr Gly Glu Tyr Arg Thr Thr Arg Pro Ile Gly Thr Arg Tyr Leu
515 520 525
Thr Arg Pro Leu
530

<210> 46
<211> 745
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 46
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Thr Leu Ser
1 5 10 15
Glu Gly Ile Arg Gln Trp Trp Lys Leu Lys Pro Gly Pro Pro Pro
20 25 30
Lys Pro Ala Glu Arg His Lys Asp Asp Ser Arg Gly Leu Val Leu Pro
35 40 45
Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60
Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80
Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
85 90 95
Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
100 105 110
Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
115 120 125
Leu Gly Leu Val Glu Glu Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140
Pro Val Glu His Ser Pro Val Glu Pro Asp Ser Ser Ser Gly Thr Gly
145 150 155 160
Lys Ala Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175
Gly Asp Ala Asp Ser Val Pro Asp Pro Gln Pro Leu Gly Gln Pro Pro
180 185 190
Ala Ala Pro Ser Gly Leu Gly Thr Asn Thr Met Ala Thr Gly Ser Gly
195 200 205
Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser
210 215 220
Ser Gly Asn Trp His Cys Asp Ser Thr Trp Met Gly Asp Arg Val Ile
225 230 235 240
Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
245 250 255
Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala Ser Asn Asp Asn His Tyr
260 265 270
Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His
275 280 285
Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn Trp
290 295 300
Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln Val
305 310 315 320
Lys Glu Val Thr Gln Asn Asp Gly Thr Thr Thr Ile Ala Asn Asn Leu
325 330 335
Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro Tyr
340 345 350
Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp
355 360 365
Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly Ser
370 375 380
Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro Ser
385 390 395 400
Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe Glu
405 410 415
Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp Arg
420 425 430

Leu	Met	Asn	Pro	Leu	Ile	Asp	Gln	Tyr	Leu	Tyr	Tyr	Leu	Ser	Arg	Thr
		435					440					445			
Asn	Thr	Pro	Ser	Gly	Thr	Thr	Thr	Gln	Ser	Arg	Leu	Gln	Phe	Ser	Gln
		450					455				460				
Ala	Gly	Ala	Ser	Asp	Ile	Arg	Asp	Gln	Ser	Arg	Asn	Trp	Leu	Pro	Gly
465					470					475					480
Pro	Cys	Tyr	Arg	Gln	Gln	Arg	Val	Ser	Lys	Thr	Ser	Ala	Asp	Asn	Asn
				485					490					495	
Asn	Ser	Glu	Tyr	Ser	Trp	Thr	Gly	Ala	Thr	Lys	Tyr	His	Leu	Asn	Gly
			500					505					510		
Arg	Asp	Ser	Leu	Val	Asn	Pro	Gly	Pro	Ala	Met	Ala	Ser	His	Lys	Asp
		515					520					525			
Asp	Glu	Glu	Lys	Phe	Phe	Pro	Gln	Ser	Gly	Val	Leu	Ile	Phe	Gly	Lys
		530				535					540				
Gln	Gly	Ser	Glu	Lys	Thr	Asn	Val	Asp	Ile	Glu	Lys	Val	Met	Ile	Thr
545					550					555					560
Asp	Glu	Glu	Glu	Ile	Arg	Thr	Thr	Asn	Pro	Val	Ala	Thr	Glu	Gln	Tyr
				565					570					575	
Gly	Ser	Val	Ser	Thr	Asn	Leu	Gln	Arg	Gly	Asn	Leu	Ala	Leu	Gly	Glu
			580					585					590		
Thr	Thr	Arg	Pro	Ala	Arg	Gln	Ala	Ala	Thr	Ala	Asp	Val	Asn	Thr	Gln
		595					600					605			
Gly	Val	Leu	Pro	Gly	Met	Val	Trp	Gln	Asp	Arg	Asp	Val	Tyr	Leu	Gln
		610				615					620				
Gly	Pro	Ile	Trp	Ala	Lys	Ile	Pro	His	Thr	Asp	Gly	His	Phe	His	Pro
625					630					635					640
Ser	Pro	Leu	Met	Gly	Gly	Phe	Gly	Leu	Lys	His	Pro	Pro	Pro	Gln	Ile
				645					650					655	
Leu	Ile	Lys	Asn	Thr	Pro	Val	Pro	Ala	Asn	Pro	Ser	Thr	Thr	Phe	Ser
			660					665					670		
Ala	Ala	Lys	Phe	Ala	Ser	Phe	Ile	Thr	Gln	Tyr	Ser	Thr	Gly	Gln	Val
		675					680					685			
Ser	Val	Glu	Ile	Glu	Trp	Glu	Leu	Gln	Lys	Glu	Asn	Ser	Lys	Arg	Trp
		690				695					700				
Asn	Pro	Glu	Ile	Gln	Tyr	Thr	Ser	Asn	Tyr	Asn	Lys	Ser	Val	Asn	Val
705					710					715					720
Asp	Phe	Thr	Val	Asp	Thr	Asn	Gly	Val	Tyr	Ser	Glu	Pro	Arg	Pro	Ile
				725					730					735	
Gly	Thr	Arg	Tyr	Leu	Thr	Arg	Asn	Leu							
			740					745							