

Adeno-Associated Virus Formulations

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial No. 63/507,266, filed June 9, 2023, the entire contents of which are incorporated herein by reference.

SEQUENCE LISTING SUBMISSION VIA EFS-WEB

[0002] A computer readable XML file, entitled "090400-5024 WO Sequence Listing" created on June 3, 2024, with a file size of about 49,743 bytes contains the sequence listing for this application and is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] It is understood that AAV formulations intended for human administration should be not only safe, sterile, and of good manufacturing practice (GMP) grade, these formulations should also exhibit and promote the long-term stability of the AAV, minimizing loss of AAV potency during the manufacture, packaging, and storage processes. Though the efforts to design such AAV formulations have been great, there still remains a need for improved AAV formulation.

SUMMARY OF THE INVENTION

[0004] Described herein are formulations compatible for human administration which address the unmet needs described above. Advantageously, in some embodiments, the formulations are suitable for long-term storage of AAV, minimizing loss of AAV potency, and advantageously prevent subvisible particle formation and prevent adsorption to the surfaces of the containers in which the AAV are packaged and stored and of the machinery used during manufacture.

[0005] In some aspects, the pharmaceutical composition of the present disclosure comprises: adeno-associated virus (AAV) and about 5 mM to about 20 mM of a buffering agent, about 100 mM to about 250 mM of a pharmaceutically acceptable salt, about 0.0001% (w/v) to about 0.01% (w/v) of a non-ionic surfactant and preferably has a pH of about 7.0 to about 9.0.

[0006] In some aspects, the pharmaceutical composition of the present disclosure comprises: AAV (e.g., AAV2 or a variant thereof), about 5 mM to about 20 mM Tris buffer, about 100 mM to about 250 mM sodium chloride, and about 0.0001% (w/v) to about 0.01% (w/v) Pluronic F68, and preferably has a pH of about 7.0 to about 9.0.

[0007] In some embodiments, the invention provides a liquid formulation. In certain embodiments, the formulation is lyophilized from a liquid formulation. In certain aspects, the lyophilized pharmaceutical composition of the present disclosure is lyophilized from a liquid formulation.

[0008] In other embodiments, methods of preparing a pharmaceutical composition comprising AAV are provided. In certain aspects, the method comprises combining about 5 mM to about 20 mM Tris buffer, about 100 mM to about 250 mM sodium chloride, about 0.0001% (w/v) to about 0.01% (w/v) Pluronic F68, and AAV, and adjusting the pH to a pH of between about 7.0 to about 9.0, thereby obtaining a pharmaceutical composition comprising AAV. Methods of storing a composition comprising AAV are also provided.

[0009] Methods for delivering a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product to a mammalian subject are provided. In exemplary aspects, the method comprises administering to the mammal a pharmaceutical composition as herein described. In some aspects, the heterologous nucleic acid is delivered to a retinal cell of the subject, e.g., a photoreceptor cell (e.g., rods; cones), a retinal ganglion cell (RGC), a glial cell (e.g., a Müller glial cell, a microglial cell), a bipolar cell, an amacrine cell, a horizontal cell, and/or a retinal pigmented epithelium (RPE) cell of the subject.

[0010] Methods of treating a subject for a disorder treatable by gene therapy are provided by the present disclosure. In exemplary aspects, the method comprises administering to the subject a pharmaceutical composition as described herein in an amount effective to treat the disorder. In some aspects, the disorder is an ocular disorder. In particularly preferred embodiments, the disorder is a VEGF-associated ocular disease such as wet or dry age-related macular degeneration (AMD) or geographic atrophy secondary to AMD.

[0011] In certain embodiments, the pharmaceutical composition of the present disclosure comprises: AAV and about 8-12 mM Tris buffer, about 160-200 mM sodium chloride, about 0.0005%-0.01% (w/v) Pluronic F68 and preferably comprises a pH of about 7.4 to 8.1. In some embodiments, the invention provides a liquid formulation. In certain embodiments, the formulation is lyophilized from a liquid formulation.

[0012] In certain embodiments, the pharmaceutical composition of the present disclosure comprises: AAV and about 8-12 mM, preferably about 10 mM, Tris buffer, about 160-200 mM, preferably about 180 mM, sodium chloride, about 0.0005%-0.01% (w/v) Pluronic F68 and comprises a pH of about 7.6. In some embodiments, the invention provides a liquid formulation. In certain embodiments, the formulation is lyophilized from a liquid formulation.

[0013] In certain embodiments, the pharmaceutical composition of the present disclosure comprises: AAV and about 8-12 mM, preferably about 10 mM, Tris buffer, about 160-200 mM, preferably about 180 mM, sodium chloride, about 0.0005%-0.01% (w/v) Pluronic F68 and comprises a pH of about 7.7. In some embodiments, the invention provides a liquid formulation. In certain embodiments, the formulation is lyophilized from a liquid formulation.

[0014] In certain embodiments, the pharmaceutical composition of the present disclosure comprises: AAV and about 8-12 mM, preferably about 10 mM, Tris buffer, about 160-200 mM, preferably about 180 mM, sodium chloride, about 0.0005%-0.01% (w/v) Pluronic F68 and comprises a pH of about 7.8. In some embodiments, the invention provides a liquid formulation. In certain embodiments, the formulation is lyophilized from a liquid formulation.

[0015] In certain embodiments, the pharmaceutical composition of the present disclosure comprises: AAV and about 8-12 mM, preferably about 10 mM, Tris buffer, about 160-200 mM, preferably about 180 mM, sodium chloride, about 0.0005%-0.01% (w/v) Pluronic F68 and comprises a pH of about 7.9. In some embodiments, the invention provides a liquid formulation. In certain embodiments, the formulation is lyophilized from a liquid formulation.

[0016] In certain embodiments, the pharmaceutical composition of the present disclosure comprises: AAV and about 8-12 mM, preferably about 10 mM, Tris buffer, about 160-200 mM, preferably about 180 mM, sodium chloride, about 0.0005%-0.01% (w/v) Pluronic F68 and

comprises a pH of about 8.0. In some embodiments, the invention provides a liquid formulation. In certain embodiments, the formulation is lyophilized from a liquid formulation.

[0017] In certain embodiments, the pharmaceutical composition of the present disclosure comprises: AAV and about 8-12 mM, preferably about 10 mM, Tris buffer, about 160-200 mM, preferably about 180 mM, sodium chloride, about 0.0005%-0.01% (w/v) Pluronic F68 and comprises a pH of about 8.1. In some embodiments, the invention provides a liquid formulation. In certain embodiments, the formulation is lyophilized from a liquid formulation.

[0018] In some embodiments, the pharmaceutical composition of the present disclosure comprises: AAV and about 9 mM to about 20 mM buffering agent; about 140 to about 200 mM pharmaceutically acceptable salt; about 0.001% (w/v) to about 0.01% (w/v) non-ionic surfactant; and a pH of about 7.3 to 8.6. In certain embodiments, the pharmaceutical composition of the present disclosure comprises: AAV and about 9-20 mM Tris buffer, about 140-200 mM sodium chloride, about 0.001%-0.01% (w/v) Pluronic F68 and comprises a pH of about 7.3 to 8.6. In some embodiments, the invention provides a liquid formulation. In certain embodiments, the formulation is lyophilized from a liquid formulation.

[0019] In certain embodiments, the pharmaceutical composition does not comprise a divalent cation and/or does not comprise a sugar or sugar alcohol.

[0020] Generally, the AAV formulations provided herein are suitable for pharmaceutical administration. In certain embodiments, the AAV is AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10. In certain embodiments, the AAV is AAV 2, AAV 5, AAV 8, or AAV 9. In certain embodiments, the AAV is AAV2 or a variant of AAV2.

[0021] In some preferred embodiments, the AAV is a recombinant AAV (rAAV) comprising a heterologous nucleic acid encoding a gene product and a capsid protein comprising a peptide insertion of from about 7 amino acids to about 20 amino acids (a “heterologous peptide” or “peptide insertion”) in the GH-loop of the capsid protein, preferably in a surface-exposed region of the GH-loop, relative to a corresponding parental AAV capsid protein. In certain embodiments, the peptide is inserted following any of the amino acids in positions 584-591 in VP1 of AAV2 or a corresponding position in another AAV serotype (i.e., the insertion site is

between amino acids 587 and 588 of VP1 of AAV2 or is between amino acids 588 and 589, between amino acids 584 and 585, between amino acids 585 and 586, between amino acids 586 and 587, between amino acids 590 and 591, or is between amino acids 591 and 592 of AAV2 or the corresponding positions in the capsid protein of another AAV serotype). In certain preferred embodiments, the insertion site is between amino acids 587 and 588 of VP1 of AAV2 or is between amino acids 588 and 589 of AAV2 or the corresponding positions in the capsid protein of another AAV serotype. In some embodiments, the capsid protein further comprises one or more amino acid substitutions relative to VP1 capsid of AAV2 or one or more corresponding substitutions in another AAV serotype, preferably wherein the capsid protein further comprises a P34A amino acid substitution relative to VP1 capsid of AAV2 or the corresponding substitution in another AAV serotype.

DESCRIPTION OF THE DRAWINGS

[0022] **Figure 1** Representative background membrane images for 3-day storage at 37°C.

[0023] **Figure 2** Aflibercept expression, from pH 6.0-7.0, after 2-week storage at 25°C/60% relative humidity (RH).

[0024] **Figure 3** Aflibercept expression, from pH 7.0-7.8, after 2-week storage at 25°C/60% relative humidity (RH).

[0025] **Figure 4** illustrates colloidal stability (> 90% Area (10-100 d.nm) by Intensity) of the tested formulations across a range of sodium chloride concentrations

[0026] **Figure 5** illustrates Final Fluorescence values by Differential Scanning Fluorimetry (DSF) at the specified sodium chloride concentrations following 0.45 µm filtration.

[0027] **Figure 6** illustrates the percentage recovery after 3 Freeze-Thaw (3XFT) by DSF at the specified sodium chloride concentrations.

DETAILED DESCRIPTION OF THE INVENTION

[0028] **Definitions**

[0029] As used herein, the term “AAV” refers to adeno-associated virus in both naturally occurring and recombinant forms (rAAV), and encompasses mutant forms of AAV. The term AAV further includes, but is not limited to, AAV type 1, AAV type 2, AAV type 3, AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, avian AAV, bovine AAV, canine AAV, equine AAV, ovine AAV, primate AAV, and non-primate AAV. In certain embodiments, the AAV is AAV2 or a variant thereof.

[0030] A “pharmaceutically acceptable salt” is a salt that can be formulated into a compound or conjugate for pharmaceutical use including, e.g., metal salts (sodium, potassium, magnesium, calcium, etc.) and salts of ammonia or organic amines that is safe for administration to a subject (e.g., a human) in a drug formulation (see, for example, Berge, et al. “Pharmaceutical Salts,” J. Pharm. Sci. 1977; 66:1, which is incorporated herein by reference in its entirety and for all purposes.). Suitable “pharmaceutically acceptable salts” include, but are not limited to, metal salts such as sodium, potassium and cesium salts; alkaline earth metal salts such as calcium and magnesium salts; organic amine salts such as triethylamine, guanidine and N-substituted guanidine salts, acetamidine and N-substituted acetamidine, pyridine, picoline, ethanolamine, triethanolamine, dicyclohexylamine, and N,N'-dibenzylethylenediamine salts. “Pharmaceutically acceptable salts” (of basic nitrogen centers) include, but are not limited to inorganic acid salts such as the hydrochloride, hydrobromide, sulfate, phosphate; organic acid salts such as trifluoroacetate and maleate salts; sulfonates such as methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, camphor sulfonate and naphthalenesulfonate; amino acid salts such as arginate, alaninate, asparagine and glutamate; and carbohydrate salts such as gluconate and galacturonate. The selection and use of pharmaceutically acceptable salts is well known in the art, for example, see Stahl and Wermuth, *Pharmaceutical Salts: Properties, Selection, and Use*, 2nd Revised edition, Wiley, Hoboken, N.J., which is incorporated herein by reference in its entirety and for all purposes. Non-limiting examples of pharmaceutically acceptable salts include, without limitation, sodium salts, ammonium salts, potassium salts (e.g, sodium, ammonium, and potassium chloride; sodium, ammonium, and potassium acetate; sodium, ammonium, and potassium citrate; sodium, ammonium, and potassium phosphate; sodium, ammonium, and potassium fluoride; sodium, ammonium, and potassium bromide; and sodium, ammonium, and potassium iodide).

[0031] The term "isolated" designates a biological material (cell, nucleic acid or protein) that has been removed from its original environment (the environment in which it is naturally present). For example, a polynucleotide present in the natural state in a plant or an animal is not isolated, however the same polynucleotide separated from the adjacent nucleic acids in which it is naturally present, is considered "isolated."

[0032] As used herein, a "coding region" or "coding sequence" is a portion of polynucleotide which consists of codons translatable into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is typically not translated into an amino acid, it can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. The boundaries of a coding region are typically determined by a start codon at the 5' terminus, encoding the amino terminus of the resultant polypeptide, and a translation stop codon at the 3' terminus, encoding the carboxyl terminus of the resulting polypeptide. Two or more coding regions can be present in a single polynucleotide construct, e.g., on a single vector, or in separate polynucleotide constructs, e.g., on separate (different) vectors. It follows, then that a single vector can contain just a single coding region, or comprise two or more coding regions.

[0033] As used herein, the term "regulatory region" refers to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding region, and which influence the transcription, RNA processing, stability, or translation of the associated coding region. Regulatory regions can include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites and stem-loop structures. If a coding region is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0034] As used herein, the term "nucleic acid" is interchangeable with "polynucleotide" or "nucleic acid molecule" and a polymer of nucleotides is intended.

[0035] A polynucleotide which encodes a gene product, e.g., a polypeptide, an interfering RNA such as a primary miRNA, short hairpin RNA (shRNA), or a small interfering RNA (siRNA), can include a promoter and/or other transcription or translation control elements

operably associated with one or more coding regions. In an operable association a coding region for a gene product, e.g., a polypeptide, is associated with one or more regulatory regions in such a way as to place expression of the gene product under the influence or control of the regulatory region(s). For example, a coding region and a promoter are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the gene product encoded by the coding region, and if the nature of the linkage between the promoter and the coding region does not interfere with the ability of the promoter to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can also be operably associated with a coding region to direct gene product expression.

[0036] "Transcriptional control sequences" refer to DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit beta-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins).

[0037] Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

[0038] The term "expression" as used herein refers to a process by which a polynucleotide produces a gene product, for example, an RNA or a polypeptide. It includes without limitation

transcription of the polynucleotide into messenger RNA (mRNA), transfer RNA (tRNA), small hairpin RNA (shRNA), small interfering RNA (siRNA) or any other RNA product, and the translation of an mRNA into a polypeptide. Expression produces a "gene product." As used herein, a gene product can be either a nucleic acid, e.g., a messenger RNA produced by transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, e.g., polyadenylation or splicing, or polypeptides with post translational modifications, e.g., methylation, glycosylation, the addition of lipids, association with other protein subunits, or proteolytic cleavage.

[0039] "Promoter" and "promoter sequence" are used interchangeably and refer to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. Promoters can be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters can direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental or physiological conditions. Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters." Promoters that cause a gene to be expressed in a specific cell type are commonly referred to as "cell-specific promoters" or "tissue-specific promoters." Promoters that cause a gene to be expressed at a specific stage of development or cell differentiation are commonly referred to as "developmentally-specific promoters" or "cell differentiation-specific promoters." Promoters that are induced and cause a gene to be expressed following exposure or treatment of the cell with an agent, biological molecule, chemical, ligand, light, or the like that induces the promoter are commonly referred to as "inducible promoters" or "regulatable promoters." It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths can have identical promoter activity.

[0040] The term "plasmid" refers to an extra-chromosomal element often carrying a gene that is not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA molecules. Such elements can be autonomously replicating sequences, genome

integrating sequences, phage or nucleotide sequences, linear, circular, or supercoiled, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell.

[0041] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same when comparing the two sequences. Sequence similarity can be determined in a number of different manners. To determine sequence identity, sequences can be aligned using the methods and computer programs, including BLAST, available over the world wide web at ncbi.nlm.nih.gov/BLAST/. Another alignment algorithm is FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wis., USA. Other techniques for alignment are described in *Methods in Enzymology*, vol. 266: *Computer Methods for Macromolecular Sequence Analysis* (1996), ed. Doolittle, Academic Press, Inc. Of particular interest are alignment programs that permit gaps in the sequence. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See *Meth. Mol. Biol.* 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. See *J. Mol. Biol.* 48: 443-453 (1970).

[0042] The term "amino acid substitution" and its synonyms described above are intended to encompass modification of an amino acid sequence by replacement of an amino acid with another, substituting, amino acid. The substitution may be a conservative substitution. It may also be a non-conservative substitution. The term conservative, in referring to two amino acids, is intended to mean that the amino acids share a common property recognized by one of skill in the art. For example, amino acids having hydrophobic nonacidic side chains, amino acids having hydrophobic acidic side chains, amino acids having hydrophilic nonacidic side chains, amino acids having hydrophilic acidic side chains, and amino acids having hydrophilic basic side chains. Common properties may also be amino acids having hydrophobic side chains, amino acids having aliphatic hydrophobic side chains, amino acids having aromatic hydrophobic side chains, amino acids with polar neutral side chains, amino acids with electrically charged side chains, amino acids with electrically charged acidic side chains, and amino acids with

electrically charged basic side chains. Both naturally occurring and non-naturally occurring amino acids are known in the art and may be used as substituting amino acids in embodiments. Methods for replacing an amino acid are well known to the skilled in the art and include, but are not limited to, mutations of the nucleotide sequence encoding the amino acid sequence. Reference to "one or more" herein is intended to encompass the individual embodiments of, for example, 1, 2, 3, 4, 5, 6, or more.

[0043] As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease (and/or symptoms caused by the disease) from occurring in a subject which may be predisposed to the disease or at risk of acquiring the disease but has not yet been diagnosed as having it; (b) inhibiting the disease (and/or symptoms caused by the disease), i.e., arresting its development; and (c) relieving the disease (and/or symptoms caused by the disease), i.e., causing regression of the disease (and/or symptoms caused by the disease), i.e., ameliorating the disease and/or one or more symptoms of the disease.

[0044] The terms "individual," "host," "subject," and "patient" are used interchangeably herein, and refer to a mammal, including, but not limited to, humans; non-human primates, including simians; mammalian sport animals (e.g., horses); mammalian farm animals (e.g., sheep, goats, etc.); mammalian pets (dogs, cats, etc.); and rodents (e.g., mice, rats, etc.).

[0045] The term "effective amount" as used herein is an amount sufficient to effect beneficial or desired clinical results. An effective amount can be administered in one or more administrations. For purposes of this disclosure, an effective amount of a compound (e.g., an infectious rAAV virion) is an amount that is sufficient to palliate, ameliorate, stabilize, reverse, prevent, slow or delay the progression of (and/or symptoms associated with) a particular disease state (e.g., a disorder associated with complement dysfunction). Accordingly, an effective amount of an infectious rAAV virion is an amount of the infectious rAAV virion that is able to effectively deliver a heterologous nucleic acid to a target cell (or target cells) of the individual.

Effective amounts may be determined preclinically by, e.g., detecting in the cell or tissue the gene product (RNA, protein) that is encoded by the heterologous nucleic acid sequence using techniques that are well understood in the art, e.g. RT-PCR, western blotting, ELISA, fluorescence or other reporter readouts, and the like. Effective amounts may be determined clinically by, e.g. detecting a change in the onset or progression of disease using methods known in the art, e.g. 6-minute walk test, left ventricular ejection fraction, hand-held dynamometry, Vignos Scale and the like as described herein and as known in the art.

Detailed Description

[0046] The present disclosure provides formulations, e.g., pharmaceutical compositions, compatible for human administration which also are suitable for long-term storage of AAV and minimizing loss of AAV potency. The formulations provided herein are advantageous, because the formulations prevent subvisible particle formation, and retain significant AAV activity when stored for extended periods of time. In certain embodiments, the pharmaceutical compositions provided herein reduce or retard degradation and/or aggregation.

[0047] In certain embodiments, the present invention provides formulations of AAV comprising a therapeutically effective amount or dose of an AAV, a pharmaceutically acceptable salt, a non-ionic surfactant, and one or more buffering agents providing a pH as herein described to the formulation. Generally, the AAV formulations provided herein are suitable for pharmaceutical administration. In certain embodiments, the AAV is AAV2 or a variant thereof.

[0048] *AAV Formulations and Compositions*

[0049] In exemplary aspects, the pharmaceutical composition of the present disclosure comprises: adeno-associated virus (AAV) and about 5 mM to about 20 mM of a buffering agent, about 100 mM to about 250 mM of a pharmaceutically acceptable salt, about 0.0001% (w/v) to about 0.01% (w/v) of a non-ionic surfactant, and a pH of at least about 7.0.

[0050] In exemplary aspects, the pharmaceutical composition of the present disclosure comprises: adeno-associated virus (AAV) and about 5 mM to about 20 mM of a buffering agent, about 100 mM to about 200 mM of a pharmaceutically acceptable salt, about 0.001% (w/v) to about 0.01% (w/v) of a non-ionic surfactant, and a pH of between about 7.0 and about 9.0.

[0051] In exemplary aspects, the pharmaceutical composition of the present disclosure comprises: adeno-associated virus (AAV) and about 5 mM to about 20 mM of a buffering agent, about 150 mM to about 200 mM of a pharmaceutically acceptable salt, about 0.001% (w/v) to about 0.01% (w/v) of a non-ionic surfactant, and a pH of at least about 7.4.

[0052] In exemplary aspects, the pharmaceutical composition of the present disclosure comprises: adeno-associated virus (AAV) and about 10 mM to about 20 mM of a buffering agent, about 150 mM to about 200 mM of a pharmaceutically acceptable salt, about 0.001% (w/v) to about 0.01% (w/v) of a non-ionic surfactant, and a pH of at least about 7.4

[0053] In certain aspects, the composition is a sterile composition. By “sterile” it is meant that there are substantially no immunogenic components in the composition, such as for example substantially no microbes (e.g., fungi, bacteria, viruses, spore forms, etc.).

[0054] In some embodiments, the invention provides a liquid formulation. In certain embodiments, the formulation is lyophilized from a liquid formulation.

[0055] In exemplary aspects, the pharmaceutical composition of the present disclosure comprises about 5 mM to about 20 mM, about 5 mM to about 15 mM, about 10 mM to about 20 mM, or about 15 mM to about 25 mM of a buffering agent. In exemplary aspects, the pharmaceutical composition comprises about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, about 20 mM, about 21 mM, about 22 mM, about 23 mM, about 24 mM, or about 25 mM of a buffering agent.

[0056] Pharmaceutically acceptable buffering agents include without limitation, phosphate buffers, histidine, sodium citrate, HEPES, Tris, Bicine, glycine, N-glycylglycine, sodium acetate, sodium carbonate, glycyl glycine, lysine, arginine, sodium phosphate, and mixtures thereof. In some preferred embodiments, the buffer is a Tris buffer.

[0057] In exemplary aspects, the pharmaceutical composition of the present disclosure comprises about 5 mM to about 25 mM, about 5 mM to about 15 mM, about 10 mM to about 20 mM, or about 15 mM to about 25 mM Tris. In exemplary aspects, the pharmaceutical composition comprises about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about

10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, about 20 mM, about 21 mM, about 22 mM, about 23 mM, about 24 mM, or about 25 mM Tris. In some embodiments, the pharmaceutical composition comprises about 8 mM Tris to about 22 mM Tris, or about 9 mM Tris to about 21 mM Tris, or about 10 mM Tris to about 20 mM Tris. In certain embodiments, the pharmaceutical composition comprises about 10 mM Tris.

[0058] In exemplary aspects, the pharmaceutical composition of the present disclosure comprises about 100 mM to about 250 mM, about 110 mM to about 240 mM, about 120 mM to about 230 mM, about 130 mM to about 220 mM, about 140 mM to about 210 mM, about 145 mM to about 205 mM, about 140 mM to about 200 mM, about 145 mM to about 200 mM, about 150 mM to about 200 mM, about 155 mM to about 195 mM, about 160 mM to about 195 mM, about 165 mM to about 195 mM, about 170 mM to about 195 mM, about 175 mM to about 195 mM, about 175 mM to about 194 mM, about 175 mM to about 193 mM, about 175 mM to about 192 mM, about 175 mM to about 191 mM, about 176 mM to about 190 mM, about 177 mM to about 189 mM, about 177 mM to about 188 mM, about 177 mM to about 187 mM, about 177 mM to about 186 mM, about 178 to about 184, about 178 to about 183, about 178 to about 182, about 179 to about 181 or about 180 mM of a pharmaceutically acceptable salt (as defined above). In exemplary embodiments, the composition comprises about 150 mM, about 155 mM, about 160 mM, about 175 mM, about 180 mM, about 185 mM, about 190 mM, or about 200 mM of a pharmaceutically acceptable salt. In certain embodiments, the pharmaceutically acceptable salt is a sodium salt (e.g., sodium chloride).

[0059] In certain embodiments, pharmaceutical compositions containing a non-ionic detergent are provided. Pharmaceutically acceptable non-ionic surfactants that may be used in the formulations disclosed herein are known in the art of pharmaceutical science, and include, without limitation, Polysorbate 80 (Tween 80; PS80), Polysorbate 20 (Tween 20; PS20), and various poloxamers or pluronics, including Pluronic F-68, and BRIJ 35, or mixtures thereof. In a preferred embodiment, the non-ionic surfactant used in the present pharmaceutical compositions is a pluronic, particularly Pluronic F-68.

[0060] In certain embodiments, the pharmaceutical composition of the present disclosure comprises about 0.001% (w/v) to about 0.01% (w/v) or about 0.0025% (w/v) to about 0.0075% (w/v) non-ionic surfactant. In exemplary aspects, the pharmaceutical composition comprises about 0.001% (w/v), about 0.0015% (w/v), about 0.002% (w/v), about 0.0025% (w/v), about 0.003% (w/v), about 0.0035% (w/v), about 0.004% (w/v), about 0.0045% (w/v), about 0.005% (w/v), about 0.0055% (w/v), about 0.006% (w/v), about 0.0065% (w/v), about 0.007% (w/v), about 0.0075% (w/v), about 0.008% (w/v), about 0.0085% (w/v), about 0.009% (w/v), about 0.0095% (w/v), about 0.001% (w/v) non-ionic surfactant. In certain embodiments, the pharmaceutical composition of the present disclosure comprises about 0.005% (w/v) non-ionic surfactant.

[0061] In exemplary aspects, the pharmaceutical composition of the present disclosure comprises about 0.001% (w/v) to about 0.01% (w/v) or about 0.0025% (w/v) to about 0.0075% (w/v) Pluronic F68. In exemplary aspects, the pharmaceutical composition comprises about 0.001% (w/v), about 0.0015% (w/v), about 0.002% (w/v), about 0.0025% (w/v), about 0.003% (w/v), about 0.0035% (w/v), about 0.004% (w/v), about 0.0045% (w/v), about 0.005% (w/v), about 0.0055% (w/v), about 0.006% (w/v), about 0.0065% (w/v), about 0.007% (w/v), about 0.0075% (w/v), about 0.008% (w/v), about 0.0085% (w/v), about 0.009% (w/v), about 0.0095% (w/v), about 0.001% (w/v) Pluronic F68. In certain embodiments, the pharmaceutical composition of the present disclosure comprises about 0.005% (w/v) Pluronic F68.

[0062] In exemplary aspects, the pharmaceutical composition of the present disclosure comprises: adeno-associated virus (AAV) and about 5 mM to about 25 mM Tris, about 100 mM to about 250 mM sodium salt, about 0.001% (w/v) to about 0.01% (w/v) non-ionic surfactant, and a pH of between 7.0 and 9.0. In certain embodiments, the pharmaceutically acceptable salt is present at about 150 nM to about 210 mM. In certain embodiments, the pharmaceutically acceptable salt is present at about 170 nM to about 200 mM. In certain embodiments, the pharmaceutically acceptable salt is sodium chloride.

[0063] The present disclosure also provides a pharmaceutical composition that can be liquid or lyophilized (e.g., lyophilized from a liquid formulation) comprising adeno-associated virus

(AAV) and about 10 mM Tris buffer, about 180 mM sodium chloride, about 0.005% (w/v) Pluronic F68 and the pH of the pharmaceutical composition is about 7.9 ± 0.3 .

[0064] pH

[0065] In exemplary embodiments, the pharmaceutical composition of the present disclosure has a pH of about 7.0, about 7.1, about 7.2, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, about 8.0, about 8.1, about 8.2, about 8.3, about 8.4, about 8.5, about 8.6, about 8.7, about 8.8, about 8.9, or about 9.0. In exemplary aspects, the pH of the pharmaceutical composition is above about 7.3 and below about 9.0 or below about 8.5. In certain embodiments, the pH of the pharmaceutical composition is about 7.8 to about 9.0 or is about 7.9. In other embodiments, the pH of the pharmaceutical composition is from about 7.3 to about 8.6.

[0066] Additional components

[0067] In some embodiments, the formulations or pharmaceutical compositions of the present disclosure comprise additional pharmaceutically acceptable ingredients. In exemplary aspects, the formulations or pharmaceutical compositions comprise any one or a combination of the following: acidifying agents, additives, adsorbents, aerosol propellants, air displacement agents, alkalizing agents, anticaking agents, anticoagulants, antimicrobial preservatives, antioxidants, antiseptics, bases, binders, buffering agents, chelating agents, coating agents, coloring agents, desiccants, detergents, diluents, disinfectants, disintegrants, dispersing agents, dissolution enhancing agents, dyes, emollients, emulsifying agents, emulsion stabilizers, fillers, film forming agents, flavor enhancers, flavoring agents, flow enhancers, gelling agents, granulating agents, humectants, lubricants, mucoadhesives, ointment bases, ointments, oleaginous vehicles, organic bases, pastille bases, pigments, plasticizers, polishing agents, preservatives, sequestering agents, skin penetrants, solubilizing agents, solvents, stabilizing agents, suppository bases, surface active agents, surfactants, suspending agents, sweetening agents, therapeutic agents, thickening agents, tonicity agents, toxicity agents, viscosity-increasing agents, water-absorbing agents, water-miscible cosolvents, water softeners, or wetting agents. In some embodiments, the formulations or pharmaceutical compositions of the present disclosure comprise any one or a combination of the following components: acacia, acesulfame potassium, acetyltributyl citrate, acetyltriethyl

citrate, agar, albumin, alcohol, dehydrated alcohol, denatured alcohol, dilute alcohol, aleuritic acid, alginic acid, aliphatic polyesters, alumina, aluminum hydroxide, aluminum stearate, amylopectin, α -amylose, ascorbic acid, ascorbyl palmitate, aspartame, bacteriostatic water for injection, bentonite, bentonite magma, benzalkonium chloride, benzethonium chloride, benzoic acid, benzyl alcohol, benzyl benzoate, bronopol, butylated hydroxyanisole, butylated hydroxytoluene, butylparaben, butylparaben sodium, calcium alginate, calcium ascorbate, calcium carbonate, calcium cyclamate, dibasic anhydrous calcium phosphate, dibasic dehydrate calcium phosphate, tribasic calcium phosphate, calcium propionate, calcium silicate, calcium sorbate, calcium stearate, calcium sulfate, calcium sulfate hemihydrate, canola oil, carbomer, carbon dioxide, carboxymethyl cellulose calcium, carboxymethyl cellulose sodium, β -carotene, carrageenan, castor oil, hydrogenated castor oil, cationic emulsifying wax, cellulose acetate, cellulose acetate phthalate, ethyl cellulose, microcrystalline cellulose, powdered cellulose, silicified microcrystalline cellulose, sodium carboxymethyl cellulose, cetostearyl alcohol, cetrimide, cetyl alcohol, chlorhexidine, chlorobutanol, chlorocresol, cholesterol, chlorhexidine acetate, chlorhexidine gluconate, chlorhexidine hydrochloride, chlorodifluoroethane (HCFC), chlorodifluoromethane, chlorofluorocarbons (CFC)chlorophenoxyethanol, chloroxylenol, corn syrup solids, anhydrous citric acid, citric acid monohydrate, cocoa butter, coloring agents, corn oil, cottonseed oil, cresol, m-cresol, o-cresol, p-cresol, croscarmellose sodium, crospovidone, cyclamic acid, cyclodextrins, dextrates, dextrin, dextrose, dextrose anhydrous, diazolidinyl urea, dibutyl phthalate, dibutyl sebacate, diethanolamine, diethyl phthalate, difluoroethane (HFC), dimethyl- β -cyclodextrin, cyclodextrin-type compounds such as Captisol®, dimethyl ether, dimethyl phthalate, dipotassium edentate, disodium edentate, disodium hydrogen phosphate, docusate calcium, docusate potassium, docusate sodium, dodecyl gallate, dodecyltrimethylammonium bromide, edentate calcium disodium, edtic acid, eglumine, ethyl alcohol, ethylcellulose, ethyl gallate, ethyl laurate, ethyl maltol, ethyl oleate, ethylparaben, ethylparaben potassium, ethylparaben sodium, ethyl vanillin, fructose, fructose liquid, fructose milled, fructose pyrogen-free, powdered fructose, fumaric acid, gelatin, glucose, liquid glucose, glyceride mixtures of saturated vegetable fatty acids, glycerin, glyceryl behenate, glyceryl monooleate, glyceryl monostearate, self-emulsifying glyceryl monostearate, glyceryl palmitostearate, glycine, glycols, glycofurol, guar gum, heptafluoropropane (HFC), hexadecyltrimethylammonium bromide, high fructose syrup, human serum albumin,

hydrocarbons (HC), dilute hydrochloric acid, hydrogenated vegetable oil type II, hydroxyethyl cellulose, 2-hydroxyethyl- β - cyclodextrin, hydroxypropyl cellulose, low-substituted hydroxypropyl cellulose, 2- hydroxypropyl- β -cyclodextrin, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, imidurea, indigo carmine, ion exchangers, iron oxides, isopropyl alcohol, isopropyl myristate, isopropyl palmitate, isotonic saline, kaolin, lactic acid, lactitol, lactose, lanolin, lanolin alcohols, anhydrous lanolin, lecithin, magnesium aluminum silicate, magnesium carbonate, normal magnesium carbonate, magnesium carbonate anhydrous, magnesium carbonate hydroxide, magnesium hydroxide, magnesium lauryl sulfate, magnesium oxide, magnesium silicate, magnesium stearate, magnesium trisilicate, magnesium trisilicate anhydrous, malic acid, malt, maltitol, maltitol solution, maltodextrin, maltol, maltose, mannitol, medium chain triglycerides, meglumine, menthol, methylcellulose, methyl methacrylate, methyl oleate, methylparaben, methylparaben potassium, methylparaben sodium, microcrystalline cellulose and carboxymethylcellulose sodium, mineral oil, light mineral oil, mineral oil and lanolin alcohols, oil, olive oil, monoethanolamine, montmorillonite, octyl gallate, oleic acid, palmitic acid, paraffin, peanut oil, petrolatum, petrolatum and lanolin alcohols, pharmaceutical glaze, phenol, liquified phenol, phenoxyethanol, phenoxypropanol, phenylethyl alcohol, phenylmercuric acetate, phenylmercuric borate, phenylmercuric nitrate, polacrillin, polacrillin potassium, poloxamer, polydextrose, polyethylene glycol, polyethylene oxide, polyacrylates, polyethylene-polyoxypropylene-block polymers, polymethacrylates, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitol fatty acid esters, polyoxyethylene stearates, polyvinyl alcohol, polyvinyl pyrrolidone, potassium alginate, potassium benzoate, potassium bicarbonate, potassium bisulfite, potassium chloride, potassium citrate, potassium citrate anhydrous, potassium hydrogen phosphate, potassium metabisulfite, monobasic potassium phosphate, potassium propionate, potassium sorbate, povidone, propanol, propionic acid, propylene carbonate, propylene glycol, propylene glycol alginate, propyl gallate, propylparaben, propylparaben potassium, propylparaben sodium, protamine sulfate, rapeseed oil, Ringer's solution, saccharin, saccharin ammonium, saccharin calcium, saccharin sodium, safflower oil, saponite, serum proteins, sesame oil, colloidal silica, colloidal silicon dioxide, sodium alginate, sodium ascorbate, sodium benzoate, sodium bicarbonate, sodium bisulfite, sodium chloride, anhydrous sodium citrate, sodium citrate dehydrate, sodium chloride, sodium cyclamate, sodium edentate, sodium dodecyl sulfate, sodium lauryl sulfate, sodium metabisulfite,

sodium phosphate, dibasic, sodium phosphate, monobasic, sodium phosphate, tribasic, anhydrous sodium propionate, sodium propionate, sodium sorbate, sodium starch glycolate, sodium stearyl fumarate, sodium sulfite, sorbic acid, sorbitan esters (sorbitan fatty esters), sorbitol, sorbitol solution 70%, soybean oil, spermaceti wax, starch, corn starch, potato starch, pregelatinized starch, sterilizable maize starch, stearic acid, purified stearic acid, stearyl alcohol, sucrose, sugars, compressible sugar, confectioner's sugar, sugar spheres, invert sugar, Sugartab, Sunset Yellow FCF, synthetic paraffin, talc, tartaric acid, tartrazine, tetrafluoroethane (HFC), theobroma oil, thimerosal, titanium dioxide, alpha tocopherol, tocopheryl acetate, alpha tocopheryl acid succinate, beta-tocopherol, delta- tocopherol, gamma-tocopherol, tragacanth, triacetin, tributyl citrate, triethanolamine, triethyl citrate, trimethyl- β -cyclodextrin, trimethyltetradecylammonium bromide, tris buffer, trisodium edentate, vanillin, type I hydrogenated vegetable oil, water, soft water, hard water, carbon dioxide-free water, pyrogen-free water, water for injection, sterile water for inhalation, sterile water for injection, sterile water for irrigation, waxes, anionic emulsifying wax, carnauba wax, cationic emulsifying wax, cetyl ester wax, microcrystalline wax, nonionic emulsifying wax, suppository wax, white wax, yellow wax, white petrolatum, wool fat, xanthan gum, xylitol, zein, zinc propionate, zinc salts, zinc stearate, or any excipient in the Handbook of Pharmaceutical Excipients, Third Edition, A. H. Kibbe (Pharmaceutical Press, London, UK, 2000), which is incorporated by reference in its entirety. Remington's Pharmaceutical Sciences, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), which is incorporated by reference in its entirety for all intended purposes, discloses various components used in formulating pharmaceutically acceptable compositions and known techniques for the preparation thereof. Except insofar as any conventional agent is incompatible with the pharmaceutical compositions, its use in pharmaceutical compositions is contemplated.

[0068] In some aspects, the formulations or pharmaceutical compositions comprise a sugar or sugar alcohol. In some aspects, the sugar or sugar alcohol is sucrose, trehalose, mannitol, or a combination thereof.

[0069] In exemplary embodiments, the formulations or pharmaceutical compositions of the present disclosure do not comprise one or a combination of the above ingredients. In exemplary embodiments, the formulations or pharmaceutical compositions of the present disclosure

comprises none of these ingredients. In exemplary aspects, the pharmaceutical composition of the present disclosure does not comprise dextran. In exemplary aspects, the pharmaceutical composition of the present disclosure does not comprise calcium chloride. In other exemplary aspects, the pharmaceutical composition of the present disclosure does not comprise a sugar or a sugar alcohol (e.g., does not comprise sucrose, trehalose, or mannitol). In related exemplary aspects, the pharmaceutical composition does not comprise glycine.

[0070] *AAV*

[0071] In exemplary embodiments, the pharmaceutical composition of the present disclosure comprises AAV. The AAV may be of any AAV serotype. In exemplary aspects, the AAV is of AAV1 serotype, AAV2 serotype, AAV3 serotype, AAV4 serotype, AAV5 serotype, AAV6 serotype, AAV7 serotype, AAV8 serotype, AAV9 serotype, or AAV10 serotype. In exemplary aspects, the AAV is of AAV2 serotype or a variant thereof.

[0072] In certain embodiments, the AAV is an rAAV as described in U.S. Patent Publication No. 2020/0282077, which is incorporated herein by reference in its entirety, in particular an rAAV comprising a capsid protein according to paragraphs 171-179 of U.S. Patent Publication No. 2020/0282077 and/or comprising a heterologous nucleic acid according to paragraphs 222-248 of U.S. Patent Publication No. 2020/0282077.

[0073] In some aspects, the pharmaceutical composition comprises an rAAV comprising a variant AAV capsid encapsulating a heterologous nucleic acid encoding gene product, wherein the variant AAV capsid comprises a variant AAV capsid protein comprising an insertion of from about 7 to about 20 amino acids (a “heterologous peptide” or “peptide insertion”) in the GH-loop of a parental AAV capsid protein, wherein the peptide comprises the amino acid sequence ISDQTKH (SEQ ID NO:1). Preferably, the variant capsid protein, when present in an AAV virion, confers increased infectivity of a retinal cell compared to the infectivity of a retinal cell by an AAV virion comprising the corresponding parental capsid protein.

[0074] By the “GH loop,” or loop IV, of the AAV capsid protein it is meant the solvent-accessible portion referred to in the art as the GH loop, or loop IV, of AAV capsid protein. For 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. Thus, for example, the insertion site can be within about amino acids 570-611 of AAV2 VP1.

[0075] In some embodiments, the peptide insertion has from 1 to 3 spacer amino acids (Y₁-Y₃) at the amino and/or carboxyl terminus of the amino acid sequence ISDQTKH (SEQ ID NO:1). Exemplary spacer amino acids include, without limitation, leucine (L), alanine (A), glycine (G), serine (S), threonine (T), and proline (P). In certain embodiments, a peptide insertion comprises 2 spacer amino acids at the N-terminus and 2 spacer amino acids at the C-terminus. In other embodiments, a peptide insertion comprises 2 spacer amino acids at the N-terminus and 1 spacer amino acids at the C-terminus. In preferred embodiments, the peptide insertion comprises or consists of the amino acid sequence LAISDQTKHA (SEQ ID NO:2).

[0076] In some aspects, the variant AAV capsid protein comprises a peptide insertion comprising the amino acid sequence ISDQTKH (SEQ ID NO:1) and further comprises one or more amino acid substitutions relative to a corresponding parental AAV capsid protein. Representative examples of amino acid substitutions may be found at e.g., col. 26, lines 40-65 of U.S. Patent No. 11,576,983, the entire contents of which are incorporated herein by reference.

[0077] In some preferred embodiments, the variant AAV capsid protein comprises a peptide insertion comprising the amino acid sequence ISDQTKH (SEQ ID NO:1) and further comprises a P34A amino acid substitution relative to VP1 capsid of AAV2 or the corresponding substitution in another AAV serotype.

[0078] In other aspects, the variant capsid protein may comprise one or more features disclosed in U.S. Patent No. 11,576,983, in particular, one or more features disclosed at column 26, line 66 to column 29, line 50 of U.S. Patent No. 11,576,983.

[0079] In a particularly preferred embodiment, the variant capsid protein comprises the following amino acid sequence or comprises an amino acid sequence at least 80%, at least 90%, least 95%, at least 98%, or at least 99% identical to the following amino acid sequence:

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPKAAERHKDDSRGLVLPGYKYLGPFNGLDKGE
PVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADADEFQERLKEDTSFGGNLGRAVFQAKKRV
LEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPQPPLG
QPPAAPSGLGTTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITSTRTWALPT

YNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFK
 LFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVVPQYGYLT
 LNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYY
 LSRTNTPSGTTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKY
 HLNGRDSLVPNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVAT
 EQYGSVSTNLQRGNLAISDQTKHARQAATADVNTQGVLPGMVWQDRDVYLQGPWAKIPHTD
 GHFHPSPLMGGFGLKHPPPQILIKNTVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENS
 KRWNPFIQYTSNYNKSVDFTVDTNGVYSEPRPIGTRYLTRNL (SEQ ID NO:3).

[0080] The variant AAV capsid protein of SEQ ID NO:3 contains the following modifications relative to native AAV2 capsid: (i) a proline (P) to alanine (A) mutation at amino acid position 34, which is located inside the assembled capsid (VP1 protein only), and (ii) an insertion of 10 amino acids (leucine-alanine-isoleucine-serine-aspartic acid-glutamine-threonine-lysine-histidine-alanine/LAISDQTKHA (SEQ ID NO:2)) at amino acid position 588, which is present in VP1, VP2, and VP3. In some embodiments, the capsid comprises a variant capsid protein comprising a sequence at least 90%, at least 95%, at least 98%, at least 99% identical to SEQ ID NO:3 and comprising a P34A substitution and an LAISDQTKHA (SEQ ID NO:2) peptide insertion at amino acid position 588.

[0081] In some aspects, a pharmaceutical composition as herein described comprises an rAAV encapsulating a heterologous nucleic acid comprising a nucleotide sequence encoding one or more gene products. In some aspects, the one or more gene products are selected from an interfering RNA (e.g., a microRNA) and a polypeptide. In particularly preferred embodiments, the heterologous nucleic acid encodes aflibercept and optionally further encodes an interfering RNA that decreases the expression of VEGF-C. In related aspects, the heterologous nucleic acid comprises a nucleotide sequence described in U.S. Patent Publication No. 2024/0131195A1, the entire contents of which are incorporated herein by reference, in particular at Table 2.

[0082] In some aspects, the rAAV comprises a heterologous nucleic acid comprising the following nucleotide sequence encoding aflibercept, codon-optimized for expression in humans:

ATGGTTTCTTACTGGGACACCGGCGTGCTGCTGTGTGCCCTGCTTTCTTGTCTGCTGCTGACC
 GGCTCTAGCAGCGGCTCTGATACCGGCAGACCCTTCGTGGAAATGTACAGCGAGATCCCCGA
 GATCATCCACATGACCGAGGGCAGAGAGCTGGTCATCCCTTGCAGAGTGACAAGCCCCAAC
 ATCACCGTGACTCTGAAGAAGTTCCTCTGGACACACTGATCCCCGACGGCAAGAGAATCAT
 CTGGGACAGCCGGAAGGGCTTCATCATCAGCAACGCCACCTACAAAGAGATCGGCCTGCTG

ACCTGTGAAGCCACCGTGAATGGCCACCTGTACAAGACCAACTACCTGACACACAGACAGA
 CCAACACCATCATCGACGTGGTGCTGAGCCCTAGCCACGGCATTGAACTGTCTGTGGGCGAG
 AAGCTGGTGCTGAACTGTACCGCCAGAACCGAGCTGAACGTGGGCATCGACTTCAACTGGG
 AGTACCCAGCAGCAAGCACCAGCACAAGAACTGGTCAACCGGGACCTGAAAACCCAGAG
 CGGCAGCGAGATGAAGAAATTCCTGAGCACCTGACCATCGACGGCGTGACCAGAAGTGAC
 CAGGGCCTGTACACATGTGCCGCCAGCTCTGGCCTGATGACCAAGAAAAACAGCACCTTCGT
 GCGGGTGACGAGAAGGACAAGACCCACACCTGTCTCCATGTCCTGCTCCAGAACTGCTCG
 GCGGACCTTCCGTGTTCTGTTTCTCCAAAGCCTAAGGACACCCTGATGATCAGCAGAACC
 CCTGAAGTGACCTGCGTGGTGGTGGATGTGTCCACGAGGATCCCGAAGTGAAGTTCAATTG
 GTACGTGGACGGCGTGGAAGTGACAACGCCAAGACCAAGCCTAGAGAGGAACAGTACAAT
 AGCACCTACAGAGTGGTGTCCGTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAAAG
 AGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCTCCTATCGAGAAAACCATCTCCAAG
 GCCAAGGGCCAGCCTAGGGAACCCCAGGTTTACACACTGCCTCCAAGCAGGGACGAGCTGA
 CAAAGAACCAGGTGTCCCTGACCTGCCTGGTCAAGGGCTTCTACCCTTCCGATATCGCCGTG
 GAATGGGAGAGCAATGGCCAGCCTGAGAACAACCTACAAGACAACCCCTCCTGTGCTGGACA
 GCGACGGCTCATCTTCTGCTACAGCAAGCTGACAGTGGACAAGAGCAGATGGCAGCAGGG
 CAACGTGTTCACTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCC
 TGAGCCTGTCTCCTGGCAAA (SEQ ID NO:4)

[0083] In some embodiments, the sequence is at least 80%, at least 90%, at least 95% or at least 99% identical to the nucleotide sequence of SEQ ID NO:4 and/or comprises a stop codon (e.g. TGA) at the end of the sequence. In some embodiments, the aflibercept gene product comprises the following amino acid sequence or a sequence at least 90%, 95%, 97%, 98%, or at least 99% identical thereto:

MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTGRELVIPCRVTSPNITVTL
 KKFPLDTLIPDGKRIIWDNRKGFIIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIIDVVLSP
 SHGIELSVGEKLVNCTARTELVNVDGIDFNWEYPSSKHQHKLVNRDLKTQSGSEMKKFLSTLTID
 GVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPSVFLFPPKPKDITLM
 SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
 GKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
 WESNGQPENNYKTTTPVLDSGDSFELYSLKLTVDKSRWQQGNVFSVMSVMHEALHNHYTQKSLSL
 SPGK (SEQ ID NO:5).

[0084] In related aspects, the pharmaceutical composition comprises an rAAV comprising a heterologous nucleic acid comprising the following sequence (encoding aflibercept + human

VEGF-C interfering RNA) or a sequence at least 80%, at least 90%, at least 95%, at least 98% or at least 99% identical thereto:

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAGGTCGCCCCGACGCC
GGGCTTTGCCCGGGCGGCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGCCCAACTCCATCACT
AGGGGTTCTATCGATTGAATTCCTCCGGGATCCACTAGTTATTAATAGTAATCAATTACGGGGTCATT
AGTTCATAGCCCATATATGGAGTTCGCGGTTACATACTTACGGTAAATGGCCCGCTGGCTGACCGC
CCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTC
CATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATAT
GCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGA
CCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGA
GCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCAACCCCAATTTTGTATTTATTTATTTT
TAATTATTTTGTGACGATGGGGGCGGGGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGG
GCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAA
AGTTTCCTTTTATGGCGAGGCGGCGGGCGGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGCGGG
GAGTGGGAGCGCGGGCGGGGGCGGTGCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGC
TGACTGACCGGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCG
CTTGTTTAAATGACGGCTTGTCTTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCC
TTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGGCGCT
CCGCGCTGCCCGGGGCTGTGAGCGCTGCGGGCGGGCGCGGGGGCTTTGTGCGCTCCGAGTGTGCGC
GAGGGGAGCGCGGGCGGGGGCGGTGCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGC
GTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTGGTGGGGCTGCAACCCCCC
TGACCCCCCTCCCCGAGTTGCTGAGCACGGCCCCGGCTTCGGGTGCGGGGCTCCGTACGGGGCGTGGC
GCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCTC
GGGCCGGGAGGGCTCGGGGAGGGGCGCGGGCGGCCCGGAGCGCCGGCGCGACTTCTTAACCCAA
CAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGGCTACCTCAGCAAGACGTTATTAGTGA
AGCCACAGATGTAAATAACGTCTTGCTGAGGTAGCTGCCTACTGCCTCGGACTTCAAGGGGCTAGAAT
TCGCGGCTGTGAGGCGCGGGCAGCCGAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGG
GACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGACCCCCCTTAGCGGG
CGCGGGGCGAAGCGGTGCGGCGCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCCGCGC
GCCGCGTCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGA
CGGGGAGGGCGGGGTTCCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCA
TGCTTCTTCTTTTCTACAGTCTAGAGTCGACCTGCAGGTGGATATCTTGGCTAGCACGCCACCATG
GTTTCTTACTGGGACACCGGCGTGTGCTGTGTGCCCTGCTTTCTTGTCTGCTGCTGACCGGCTCTAGC
AGCGGCTCTGATACCGGCAGACCCTTCGTGGAATGTACAGCGAGATCCCCGAGATCATCCACATGAC
CGAGGGCAGAGAGCTGGTCATCCCTTGACAGAGTGACAAGCCCCAACATCACCGTGACTCTGAAGAAG
TTCCCTCTGGACACACTGATCCCCGACGGCAAGAGATCATCTGGGACAGCCGGAAGGGCTTCATCAT
CAGCAACGCCACCTACAAAGAGATCGGCTGTGACCTGTGAAGCCACCGTGAATGGCCACCTGTACA
AGACCAACTACCTGACACACAGACAGACCAACACCATCATCGACGTGGTGTGAGCCCTAGCCACGG
CATTGAAGTGTCTGTGGGCGAGAAGCTGGTGTGAACTGTACCGCCAGAACCAGCTGAACGTGGGCA
TCGACTTCAACTGGGAGTACCCAGCAGCAAGCACCAGCACAAGAACTGGTCAACCGGGACCTGAA
AACCAGAGCGGCAGCGAGATGAAGAAATTCCTGAGCACCTGACCATCGACGGCGTGACCAGAAGT
GACCAGGGCCTGTACACATGTCCCGCAGCTCTGGCCTGATGACCAAGAAAAACAGCACCTTCGTGCG
GGTGACAGAGAAGGACAAGACCCACACCTGTCTCTCATGTCTGCTCCAGAACTGCTCGGCGGACCTT
CCGTGTTCTGTTTCTCCAAAGCCTAAGGACACCCTGATGATCAGCAGAACCCTGAAGTGACCTGC
GTGGTGGTGGATGTGTCCACGAGGATCCCGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGT
GCACAACGCCAAGACCAAGCCTAGAGAGGAACAGTACAATAGCACCTACAGAGTGGTGTCCGTGCTG
ACCGTGCTGACACAGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGC
CTGCTCTATCGAGAAAACCATCTCCAAGGCCAAGGGCCAGCCTAGGGAACCCAGGTTTACACACTG
CCTCCAAGCAGGGACGAGCTGACAAAGAACCAGGTGTCCCTGACCTGCCTGGTCAAGGGCTTCTACCC
TTCCGATATCGCCGTGGAATGGGAGAGCAATGGCCAGCCTGAGAACAACATAAGACAACCCCTCTG
TGCTGGACAGCGACGGCTCATTCTTCTGTACAGCAAGCTGACAGTGGAACAAGAGCAGATGGCAGCA
GGGCAACGTGTTAGCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGA
GCCTGTCTCCTGGCAAATGAGCCACGCGTAACAGTGGGGGATCCAGACATGATAAGATACATTGATG

AGTTTGGACAAACCACAACCTAGAAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATT
GCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTT
CAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGCTGA
TTATGATCAATGCATGGCCGGCCGGAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGC
TCGCTCGCTCACTGAGGCCGCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAG
TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA (SEQ ID NO:6).

[0085] In related aspects, the pharmaceutical composition comprises an rAAV comprising a heterologous nucleic acid comprising a nucleotide sequence encoding a wild type or codon-optimized human Rab escort protein-1 (REP1) protein, e.g., as described in U.S. Patent No. 11,357,870, the entire contents of which are hereby incorporated by reference. In some preferred aspects, the heterologous nucleic acid encoding REP1 comprises the following codon-optimized nucleotide sequence or a sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98% or at least 99% identical thereto:

ATGGCTGATACACTGCCTTCTGAGTTTGATGTGATCGTGATTGGAAGTGGACTGCCTGAGAG
TATTATTGCTGCTGCTTGTAGTAGAAGCGGCCGGAGAGTGCTGCACGTGGACAGCAGATCCT
ACTATGGCGGCAACTGGGCCTCTTTCAGCTTTTCCGGCCTGCTGAGCTGGCTGAAGGAGTAC
CAGGAGAACTCCGACATCGTGTCTGATAGCCCCGTGTGGCAGGACCAGATCCTGGAGAATG
AGGAGGCCATCGCCCTGTCCAGGAAGGATAAGACCATCCAGCACGTGGAGGTGTTCTGCTA
TGCCAGCCAGGACCTGCACGAGGATGTGGAGGAGGCAGGCGCCCTGCAGAAGAACCACGCC
CTGGTGACCTCCGCCAATTCTACAGAGGCCGCCGACTCCGCCTTTCTGCCTACCGAGGATGA
GTCCCTGTCTACAATGTCTTGTGAGATGCTGACCGAGCAGACACCTAGCTCCGATCCAGAGA
ACGCCCTGGAGGTCAATGGCGCCGAGGTGACCGGCGAGAAGGAGAACCACTGCGACGATAA
GACCTGCGTGCCAAGCACATCCGCCGAGGACATGTCCGAGAACGTGCCTATCGCCGAGGAT
ACCACAGAGCAGCCAAAGAAGAATCGCATCACATACAGCCAGATCATCAAGGAGGGCAGG
CGCTTCAATATCGACCTGGTGTCTAAGCTGCTGTACAGCCGGGGCCTGCTGATCGATCTGCT
GATCAAGAGCAACGTGTCCCGCTATGCCGAGTTCAAGAATATCACCAGAACTCTGGCCTTTC
GGGAGGGAAGAGTGGAGCAGGTGCCCTGCAGCAGAGCCGACGTGTTCAACTCCAAGCAGCT
GACAATGGTGGAGAAGAGGATGCTGATGAAGTTCCTGACATTTTGTATGGAGTACGAGAAG
TATCCAGATGAGTACAAGGGCTATGAGGAGATCACCTTTTACGAGTATCTGAAGACCCAGA
AGCTGACACCCAATCTGCAGTACATCGTGTGCACTCCATCGCCATGACCTCTGAGACAGCC
TCTAGCACCATCGACGGCCTGAAGGCCACAAAGAACTTCCTGCACTGCCTGGGCCGGTACGG
CAATACACCCCTTCTGTTTCTCTGTATGGCCAGGGCGAGCTGCCCCAGTGCTTCTGTAGAAT
GTGCGCCGTGTTTGGCGGCATCTATTGCCTGAGGCACTCTGTGCAGTGTCTGGTGGTGGACA
AGGAGAGCCGCAAGTGTAAGGCCATCATCGATCAGTTTGGCCAGCGGATCATCTCTGAGCA

CTTCCTGGTGGAGGACAGCTACTTTCTGAGAACATGTGCTCCAGGGTGCAGTATCGCCAGA
TCAGCCGGGGCCGTGCTGATCACCGATAGATCCGTGCTGAAGACAGACAGCGATCAGCAGAT
CAGCATCCTGACCGTGCCAGCAGAGGAGCCAGGCACCTTCGCCGTGAGAGTGATCGAGCTG
TGCTCCTCTACCATGACATGTATGAAGGGCACCTACCTGGTGCACCTGACCTGCACAAGCTC
CAAGACAGCCCCGCGAGGACCTGGAGAGCGTGGTGCAGAAGCTGTTCGTGCCCTACACCGAG
ATGGAGATCGAGAACGAGCAGGTGGAGAAGCCTAGAATCCTGTGGGGCCCTGTACTTCAACA
TGAGAGACTCTAGCGATATCTCTAGGAGCTGTTACAACGATCTGCCCTCTAACGTGTACGTG
TGCAGCGGACCTGACTGTGGCCTGGGAAACGATAATGCCGTGAAGCAGGCCGAGACACTGT
TCCAGGAGATTTGCCCTAACGAGGACTTTTGTCCCCCTCCACCCAATCCAGAGGATATCATC
CTGGACGGCGATTCCCTGCAGCCAGAGGCCTCTGAGTCCTCTGCCATCCCCGAGGCCAATAG
CGAAACATTCAAAGAAAGCACAAATCTGGGAAACCTGGAAGAAAGTAGTGAGTAA (SEQ ID
NO:7)

[0086] In other related aspects, the pharmaceutical composition comprises an rAAV comprising a heterologous nucleic acid comprising a nucleotide sequence encoding a wild type or codon-optimized human Retinitis Pigmentosa GTPase Regulator (*RPGR*) protein, e.g., as described in U.S. Patent No. 11,345,930, the entire contents of which are hereby incorporated by reference. In some preferred aspects, the heterologous nucleic acid encodes human RPGR ORF15 and comprises the following codon-optimized nucleotide sequence or a sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 97%, at least 98% or at least 99% identical thereto:

ATGAGAGAACCCGAGGAACTGATGCCCCACTCTGGCGCCGTGTTTACCTTCGGCAAGAGCAAGTT
CGCCGAGAACAACCCCGGCAAGTTCTGTTTCAAGAACGACGTGCCAGTGACCTGAGCTGCGGA
GATGAACACTCTGCCGTGGTCACCGGCAACAACAAGCTGTACATGTTTCGGCAGCAACAACCTGGG
GCCAGCTCGGCCTGGGATCTAAGTCTGCCATCAGCAAGCCTACCTGCGTGAAGGCCCTGAAGCCT
GAGAAAGTGAACTGGCCGCCTGCGGCAGAAATCACACCCTGGTTTCTACCGAAGGCGGCAATG
TGTATGCCACCGGCGGAAACAATGAGGGACAGCTTGGACTGGGCGACACCGAGGAAAGAAACA
CCTTCCACGTGATCAGCTTTTTACACGCGAGCACAAAGATCAAGCAGCTGAGCGCCGGCTCTAAT
ACCTCTGCCGCTCTGACAGAGGACGGCAGACTGTTTATGTGGGGCGACAATTCTGAGGGCCAGAT
CGGACTGAAGAACGTGTCCAATGTGTGCGTGCCCCAGCAAGTGACAATCGGCAAGCCTGTGTCTT
GGATCAGCTGCGGCTACTACCACAGCGCCTTTGTGACAACCGATGGCGAGCTGTATGTGTTTCGGC
GAGCCAGAGAATGGCAAGCTGGGACTGCCTAACAGCTGCTGGGCAATCACAGAACCCCTCAGC
TGGTGTCTGAGATCCCCGAAAAAGTGATCCAGGTGGCCTGTGGCGGAGAGCACACAGTGGTGCT
GACAGAGAATGCCGTGTACACCTTTGGCCTGGGCCAGTTTGGACAACCTCGGACTGGGAACCTTCC

TGTTTCGAGACAAGCGAGCCCAAAGTGATCGAGAACATCCGGGACCAGACCATCAGCTACATCAG
CTGTGGCGAGAACCACACAGCCCTGATCACAGACATCGGCCTGATGTACACATTGCGCGACGGA
AGGCATGGAAAGCTCGGACTTGCCCTGGAAAACCTCACCAACCACTTCATCCCTACGCTGTGCAG
CAACTTCCTGCGGTTTCATTGTGAAGCTGGTGGCCTGCGGAGGATGCCACATGGTGGTTTTTGCTG
CCCCTCACAGAGGCGTGGCCAAAGAGATTGAGTTCGACGAGATCAACGATACCTGCCTGAGCGT
GGCCACCTTCCTGCCTTACAGCAGCCTGACATCTGGCAACGTGCTGCAGAGGACACTGAGCGCCA
GAATGCGCAGACGGGAAAGAGAGAGAAGCCCCGACAGCTTCAGCATGAGAAGAACCCTGCCTCC
AATCGAGGGCACACTGGGCCTGTCTGCCTGCTTTCTGCCTAACAGCGTGTTCCCCAGATGCAGCG
AGAGAAACCTGCAAGAGAGCGTGCTGAGCGAGCAGGATCTGATGCAGCCTGAGGAACCCGACTA
CCTGCTGGACGAGATGACCAAAGAGGGCCGAGATCGACAACAGCAGCACAGTGGAAGCCTGGG
CGAGACAACCGACATCCTGAACATGACCCACATCATGAGCCTGAACAGCAACGAGAAGTCTCTG
AAGCTGAGCCCCGTGCAGAAGCAGAAGAAGCAGCAGACCATCGGCGAGCTGACACAGGATACT
GCCCTGACCGAGAACGACGACAGCGACGAGTACGAAGAGATGAGCGAGATGAAGGAAGGCAAG
GCCTGCAAGCAGCACGTGTCCCAGGGCATCTTTATGACCCAGCCTGCCACCACCATCGAGGCCTT
TTCCGACGAGGAAGTGGAATCCCCGAGGAAAAAGAGGGGCGCCGAGGACAGCAAAGGCAACGG
CATTGAGGAACAAGAGGTGGAAGCCAACGAAGAGAACGTGAAGGTGCACGGCGGACGGAAAGA
AAAGACCGAGATCCTGAGCGACGACCTGACCGATAAGGCCGAGGTTTCCGAGGGCAAAGCCAAG
TCTGTGGGAGAAGCCGAGGATGGACCTGAAGGCCGCGGAGATGGAACCTGTGAAGAAGGATCTA
GCGGAGCCGAGCACTGGCAGGATGAGGAACCGGAGAAGGGCGAGAAAGACAAAGGCAGAGGC
GAGATGGAAGACCCGCGAGGGCGAAAAAGAGCTGGCCGAGAAAGAGGAATGGAAGAAACG
CGACGGCGAAGAACAAGAGCAGAAAGAAAGAGAGCAGGGCCACCAGAAAGAACGGAATCAAG
AGATGGAAGAAGGCGGCGAGGAAGAACCGGCGAAGGGGAAGAAGAGGAAGGCGACCGAGAG
GAAGAAGAAGAGAAAGAAGGCGAAGGCAAAGAAGAAGGCGAGGGCGAAGAGGTGGAAGGCGA
GCGTGAAAAAGAAGAGGGCGAACGCAAGAAAGAAGAACCGCGCCGAAAAGAGGAAAAAGGCG
AGGAAGAGGGCGACCAAGGCGAAGGCGAGGAAGAAGAACTGAAGGCAGAGGGGAAGAGAAA
GAGGAAGGCGGCGAAGTCAAGGCGGAGAGGTTGAAGAAGGCAAAGGCGAGCGAGAAGAGGA
AGAAGAAGAAGGCGAAGGCGAGGAAGAGGAAGGCGAAGGCGAAGAGGAAGAAGGCGAAGGGG
AAGAAGAAGAAGGCGAAGGCAAGGGCGAAGAGGAGGGCGAAGAAGGCGAGGGCGAAGAGGAG
GGCGAAGAAGGCGAAGGCGAGGGCGAAGAAGAAGAAGGCGAAGGCGAAGGCGAGGAAGAAGG
CGAAGGCGAAGGGGAAGAAGAGGAAGGCGAAGGCGAAGGCGAAGAAGAAGGCGAAGGCGAGG
GCGAAGAGGAAGAAGGCGAAGGCAAAGGGGAAGAAGAAGGCGAGGAAGGCGAAGGCGAAGGC
GAGGAAGAAGAAGGCGAAGGCGAGGGCGAAGATGGCGAAGGCGAAGGCGAAGAGGAAGAGGG
CGAGTGGGAGGGCGAAGAAGAGGAAGGCGAAGGCGAGGGCGAAGAGGAAGGCGAAGGCGAGG
GCGAAGAAGGCGAAGGCGAAGGCGAGGAAGAGGAAGGCGAAGGCGAAGGGGAAGAAGAAGAG
GGCGAAGAAGAAGGCGAAGAGGAAGGCGAAGGGGAAGAAGAAGGCGAAGGCGAAGGCGAAGA
AGAGGAAGAGGGCGAAGTTGAAGGCGAGGTTGAGGGCGAAGAAGGCGAAGGCGAAGGGGAAG

AAGAAGAAGGCGAGGAAGAAGGGGAAGAGAGAGAAAAAGAAGGCGAGGGCGAAGAAAACCGC
CGGAACCGCGAAGAGGAAGAGGAAGAAGAGGGCAAGTACCAAGAGACTGGCGAGGAAGAGAA
CGAGCGGCAGGATGGCGAAGAGTACAAGAAGGTGTCCAAGATCAAGGGCAGCGTGAAGTACGG
CAAGCACAAGACCTACCAGAAGAAGTCCGTCACCAACACGCAAGGCAATGGAAAAGAACAGCG
GAGCAAGATGCCCCGTGCAGTCCAAGAGGCTGCTGAAGAATGGCCCTAGCGGCAGCAAGAAATTC
TGGAACAATGTGCTGCCCCACTACCTCGAGCTGAAGTGA (SEQ ID NO:8)

[0087] In other related aspects, the pharmaceutical composition comprises an rAAV comprising a heterologous nucleic acid comprising a nucleotide sequence encoding an engineered human complement regulator factor H (fH) gene operably linked to an expression control sequence, wherein the human fH (hfH) gene encodes a soluble hfH protein variant that retains complement regulatory function, wherein said fH variant comprises short consensus repeats (SCRs) 1, 2, 3, 4, 19 and 20. In some aspects, the soluble hfH protein variant comprises an amino acid sequence as set forth in U.S. Patent No. 10,988,519, the entire contents of which are incorporated herein by reference. In a particularly preferred embodiment, the heterologous nucleic acid encodes an fH variant having the following amino acid sequence:

MRLLAKIICLMLWAICVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPGYRSLG
NIIMVCRKGEWVALNPLRKCKQKRPCGHPGDTFPGTFTLTGGNVFEYGVKAVYTCNEGY
QLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENGKIVSSAMEPDREYHFGQAVRFV
CNSGYKIEGDEEMHCSDDGFWWSKEKPKCVEISCKSPDVINGSPISQKIIYKENERFQYKC
NMGYEYSERGDAVCTESGWRPLPSCEEKSTLKPCDYPDIKHGGLYHENMRRPYFPVAV
GKYYSYCYCDEHFETPSGSYWDHIHCTQDGWSPAVPCLRKCYFPYLENGYNQNYGRKF
VQGKSIDVACHPGYALPKAQTTVTCMENGWSPTPRCIRVKTCSKSSIDIENGFISESQYT
YALKEKAKYQCKLGYVTADGETSGSITCGKDGWSAQPTCIKSIKTDCLSLPSFENAI PMG
EKKDVYKAGEQVTYTCATYYKMDGASNVTICNSRWTGRPTCRDTSVNPPTVQNAYI
VSRQMSKYPSEGERVRYQCRSPYEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPIDN
GDITSFPLSVYAPASSVEYQCQNLYQLEGNKRITCRNGQWSEPPKCLHPCVISREIMENY
NIALRWTAKQKLYSRTGESVEFVCKRGYRLSSRSHTLR TTCWDGKLEYPTCAKR (SEQ
ID NO:9)

[0088] In some aspects, the heterologous nucleic acid comprises the following nucleotide sequence or a nucleotide sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical thereto:

TTGGCCACTCCCTCTCTGCGGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTC
GCCCCGACGCCCGGGCTTTGCCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCTATCGATTGAATTCCTCGGGGATCCA
CTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGT

TCCGCGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCCC
GCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAG
TGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCT
GGCATTATGCCCAGTACATGACCTTATGGGACTTTCTTACTTGGCAGTACATCTACG
TATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCC
CATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTAAATTATTTTGTG
CAGCGATGGGGGCGGGGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGG
GCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGG
CGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAA
AGCGAAGCGCGCGGGCGGGGAGTCTGCTGCGACGCTGCCTTCGCCCCGTGCCCC
GCTCCGCCGCCGCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCAC
AGGTGAGCGGGCGGGACGGCCCTTCTCTCCGGGCTGTAATTAGCGCTTGGTTAAT
GACGGCTTGTTCCTTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCC
CTTTGTGCGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGCGTGCGGGAGC
GCCGCGTGCGGCTCCGCGCTGCCGCGGGCTGTGAGCGCTGCGGGCGCGGCGCGGG
GCTTTGTGCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCGC
GGGTGAGCAGGGGGTGTGGGCGCGTCCGGTCCGGGCTGCAACCCCCCTGCACCCCC
CTCCCCGAGTTGCTGAGCACGGCCCCGGCTTCGGGTGCGGGGCTCCGTACGGGGCGT
GGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCG
GGGCGGGGCGCCCTCGGGCCGGGGAGGGCTCGGGGAGGGGCGCGGCGGCCCCCCG
GAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAAT
CGTGCGAGAGGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTG
GGAGGCGCCGCCGACCCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGC
AGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCCTCT
CCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGACGGG
GCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACC
ATGTTTATGCCTTCTTCTTTTCTTACAGTCTAGAGTCGACCTGCAGGTGGATATCTT
GCTAGCACGCCACCATGCGCCTGCTGGCCAAGATCATCTGCCTGATGCTGTGGGCCA
TCTGCGTGCGCGAGGACTGTAACGAGCTGCCCCCTCGGAGAAATACAGAGATCCTG
ACCGGCTCTTGAGCGATCAGACCTACCCAGAGGGCACACAGGCCATCTACAAGTG
CAGGCCCCGGCTATCGCTCCCTGGGCAATATCATCATGGTGTGCAGGAAGGGAGAGT
GGGTGGCCCTGAACCCACTGAGAAAGTGCCAGAAGAGGCCATGTGGACACCCAGGC
GACACACCTTTTCGGCACCTTTACACTGACCGGCGGCAACGTGTTTCGAGTACGGCGTG
AAGGCCGTGTATACCTGCAATGAGGGCTACCAGCTGCTGGGCGAGATCAACTACCG
GGAGTGTGACACAGATGGCTGGACCAATGATATCCCCATCTGCGAGGTGGTGAAGT
GTCTGCCAGTGACCGCCCCCGAGAACGGCAAGATCGTGAGCTCCGCCATGGAGCCT
GACAGAGAGTATCACTTCGGCCAGGCCGTGAGGTTCTGTGTGCAATAGCGGCTACAA
GATCGAGGGCGATGAGGAGATGCACTGTTCCGACGATGGCTTCTGGTCTAAGGAGA
AGCCCAAGTTCGTGGAGATCTCCTGTAAGTCTCCTGACGTGATTAACGGCAGCCCAA
TCTCCCAGAAGATCATCTATAAGGAGAATGAGCGGTTTCAGTACAAGTGCAACATG
GGCTACGAGTATTCCGAGAGAGGGCGATGCCGTGTGCACAGAGTCTGGATGGAGGCC
CCTGCCTAGCTGCGAGGAGAAGTCCACCTGAAGCCTTGTGACTATCCAGATATCAA
GCACGGCGGCCTGTATCACGAGAATATGAGGCGCCCTTACTTCCAGTGCCCGTGG
GCAAGTACTATTCTACTATTGCGACGAGCACTTTGAGACACCATCCGGCTCTTACT

GGGACCACATCCACTGTACCCAGGATGGCTGGTCTCCCGCCGTGCCTTGCCTGAGAA
AGTGTTACTTCCCCTATCTGGAGAACGGCTACAACCAGAATTATGGCAGGAAGTTTG
TGCAGGGCAAGTCCATCGACGTGGCATGCCACCCAGGATACGCACTGCCTAAGGCA
CAGACCACAGTGACCTGTATGGAGAATGGCTGGAGCCCAACACCCAGGTGCATCCG
CGTGAAGACCTGTTCCAAGAGCAGCATCGACATCGAGAACGGCTTCATCAGCGAGT
CCCAGTACACCTATGCCCTGAAGGAGAAGGCCAAGTATCAGTGCAAGCTGGGATAC
GTGACCGCAGACGGAGAAACATCTGGCAGCATCACCTGCGGCAAGGATGGCTGGTC
TGCCCAGCCCACATGCATCAAGAGCATCAAGACCGACTGTCTGTCTCTGCCAGCTT
TGAGAATGCCATCCCTATGGGCGAGAAGAAGGACGTGTACAAGGCCGGCGAGCAGG
TGACATACACCTGCGCCACATACTATAAGATGGACGGCGCCAGCAACGTGACCTGT
ATCAATTCCCGGTGGACAGGCAGACCTACCTGCAGGGATACAAGCTGCGTGAACCC
ACCCACCGTGCAGAATGCCTATATCGTGTCCCGCCAGATGTCTAAGTACCCTAGCGG
CGAGCGGGTGAGATATCAGTGCCGGTCCCCATACGAGATGTTCCGGCGACGAGGAAG
TGATGTGCCTGAACGGCAATTGGACAGAGCCTCCACAGTGCAAGGATAGCACCGGC
AAGTGTGGACCACCTCCACCAATCGACAACGGCGATATCACATCCTTTCCACTGAGC
GTGTACGCCCCCGCCTCCTCTGTGGAGTATCAGTGCCAGAACCTGTACCAGCTGGAG
GGCAATAAGCGCATCACCTGTGGAACGGCCAGTGGTCTGAGCCTCCAAAGTGCCT
GCACCCTTGCCTGATCTCCAGAGAGATCATGGAGAACTATAATATCGCCCTGCGCTG
GACAGCCAAGCAGAAGCTGTACTCTCGGACCGGCGAGAGCGTGGAGTTCGTGTGCA
AGCGGGGCTATAGACTGAGCTCCCGCAGCCACACACTGCGGACCACATGTGGGAC
GGCAAGCTGGAGTACCCAACCTGTGCCAAGAGGTGAGCCACGCGTAACACGTGCAT
GCGAGAGATGTGCGGCGCGAGCTCGGGGATCCAGACATGATAAGATACATTGATG
AGTTTGACAAACCACAACACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTT
GTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACA
ACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTTAAA
GCAAGTAAACCTCTACAAATGTGGTATGGCTGATTATGATCAATGCATCCTAGCCG
GAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACT
GAGGCCGCCCGGGCAAAGCCCGGGCGTTCGGGCGACCTTTGGTCGCCCCGGCCTCAGT
GAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA (SEQ ID NO:10)

[0089] In related aspects, the heterologous nucleic acid comprises the following nucleotide sequence or a nucleotide sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical thereto:

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTC
GCCCGACGCCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCTATCGATTGAACCAGGCGCGCCAG
TTTAAACAGTCCGGAAGTATTATTAATAGTAATCAATTACGGGGTCATTAGTTCAT
AGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGA
CCGCCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACG
CCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCAC
TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGAC
GGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCTACT
TGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACG
TTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTGTATTTATTTAT

TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGGGCGCGCGCCAG
GCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGG
CAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGG
CGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGGAGTCGCTGCGACGCTGC
CTTCGCCCCGTGCCCCGCTCCGCCGCCGCTCGCGCCGCCCGCCCCGGCTCTGACTG
ACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCTCCGGGCTGTAAT
TAGCGCTTGGTTTAATGACGGCTTGTTCCTTTTCTGTGGCTGCGTGAAAGCCTTGAGG
GGCTCCGGGAGGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGT
GTGTGCGTGGGGAGCGCCGCGTGC GGCTCCGCGCTGCCCGGCGGCTGTGAGCGCTG
CGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCG
GGGGCGGTGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCG
GGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCGGTCGGGCTGCAAC
CCCCCTGCACCCCCCTCCCCAGTTGCTGAGCACGGCCCCGGCTTCGGGTGCGGGGC
TCCGTACGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTG
GGGGTGCCGGGCGGGGCGGGGCCGCTCGGGCCGGGGAGGGCTCGGGGGAGGGGC
GCGGCGGCCCCCGAGCGCCGGCGGCTGTGAGGCGCGGCGAGCCGCAGCCATTGC
CTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCAAATCTGTGCG
GAGCCGAAATCTGGGAGGCGCCGCCGACCCCCCTCTAGCGGGCGCGGGGCGAAGCG
GTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCCTCGTGCGTCGCCGCGCC
GCCGTCCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTC
GGGGGGGACGGGGCAGGGCGGGGTTCTGGCTTCTGCGGTGTGACCGGGCTCTAGA
GCCTTGCTAACCATGTTTCATGCCTTCTCTTTTCTACAGTCTAGAGTCGACGGTC
ACCGGTAGTACGACGCCACCATGCGCCTGTGCGCAAGATCATCTGCCTGATGCT
GTGGGCCATCTGCGTGGCCGAGGACTGTAAAGAGCTGCCCCCTCGGAGAAATACAG
AGATCCTGACCGGCTCTTGAGCGATCAGACCTACCCAGAGGGCACACAGGCCATC
TACAAGTGCAGGCCCCGCTATCGCTCCCTGGGCAATATCATCATGGTGTGCAGGAAG
GGAGAGTGGGTGGCCCTGAACCCACTGAGAAAGTGCCAGAAGAGGCCATGTGGACA
CCCAGGCGACACACCTTTCGGCACCTTTACACTGACCGGCGGCAACGTGTTGAGTA
CGGCGTGAAGGCCGTGTATACCTGCAATGAGGGCTACCAGCTGCTGGGCGAGATCA
ACTACCGGGAGTGTGACACAGATGGCTGGACCAATGATATCCCCATCTGCGAGGTG
GTGAAGTGTCTGCCAGTGACCGCCCCCGAGAACGGCAAGATCGTGAGCTCCGCCAT
GGAGCCTGACAGAGAGTATCACTTCGGCCAGGCCGTGAGGTTCGTGTGCAATAGCG
GCTACAAGATCGAGGGCGATGAGGAGATGCACTGTTCCGACGATGGCTTCTGGTCT
AAGGAGAAGCCCAAGTGCGTGGAGATCTCCTGTAAGTCTCCTGACGTGATTAACGG
CAGCCCAATCTCCCAGAAGATCATCTATAAGGAGAATGAGCGGTTTCAGTACAAGT
GCAACATGGGCTACGAGTATTCCGAGAGAGGCGATGCCGTGTGCACAGAGTCTGGA
TGGAGGCCCCCTGCCTAGCTGCGAGGAGAAGTCCACCCTGAAGCCTTGTGACTATCCA
GATATCAAGCACGGCGGCCTGTATCACGAGAATATGAGGCGCCCTTACTTCCCAGTG
GCCGTGGGCAAGTACTATTCTACTATTGCGACGAGCACTTTGAGACACCATCCGGC
TCTTACTGGGACCACATCCACTGTACCCAGGATGGCTGGTCTCCCGCCGTGCCTTGC
CTGAGAAAGTGTTACTTCCCCTATCTGGAGAACGGCTACAACCAGAATTATGGCAGG
AAGTTTGTGCAGGGCAAGTCCATCGACGTGGCATGCCACCCAGGATACGCACTGCC
TAAGGCACAGACCACAGTGACCTGTATGGAGAATGGCTGGAGCCCAACACCCAGGT
GCATCCGCGTGAAGACCTGTTCCAAGAGCAGCATCGACATCGAGAACGGCTTCATC
AGCGAGTCCCAGTACACCTATGCCCTGAAGGAGAAGGCCAAGTATCAGTGCAAGCT
GGGATACGTGACCGCAGACGGAGAAACATCTGGCAGCATCACCTGCGGCAAGGATG

GCTGGTCTGCCCAGCCCACATGCATCAAGAGCATCAAGACCGACTGTCTGTCTCTGC
CCAGCTTTGAGAATGCCATCCCTATGGGCGAGAAGAAGGACGTGTACAAGGCCGGC
GAGCAGGTGACATACACCTGCGCCACATACTATAAGATGGACGGCGCCAGCAACGT
GACCTGTATCAATTCCCGGTGGACAGGCAGACCTACCTGCAGGGATACAAGCTGCG
TGAACCCACCCACCGTGCAGAAATGCCTATATCGTGTCCCGCCAGATGTCTAAGTACC
CTAGCGGCGAGCGGGTGAGATATCAGTGCCGGTCCCCATACGAGATGTTCCGGCGAC
GAGGAAGTGATGTGCCTGAACGGCAATTGGACAGAGCCTCCACAGTGCAAGGATAG
CACCGGCAAGTGTGGACCACCTCCACCAATCGACAACGGCGATATCACATCCTTTCC
ACTGAGCGTGTACGCCCCCGCCTCCTCTGTGGAGTATCAGTGCCAGAACCTGTACCA
GCTGGAGGGCAATAAGCGCATCACCTGTCCGAACGGCCAGTGGTCTGAGCCTCCAA
AGTGCCTGCACCCTTGCGTGATCTCCAGAGAGATCATGGAGAACTATAATATCGCCC
TGCGCTGGACAGCCAAGCAGAAGCTGTACTCTCGGACCGGCGAGAGCGTGGAGTTC
GTGTGCAAGCGGGGCTATAGACTGAGCTCCCGCAGCCACACACTGCGGACCACATG
CTGGGACGGCAAGCTGGAGTACCCAACCTGTGCCAAGAGGTGAGCCTAGGACTGTA
CAGTACGCGTAACACGTGGGGGATCCAGACATGATAAGATAACATTGATGAGTTTGG
ACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGC
TATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTG
CATTCATTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTA
AAACCTCTACAAATGTGGTATGGCTGATTATGATCAATGCATGGTCCCAGCCTTCAT
TGAGCCTATTGGTCCCCCCTCAGAACCCACCCACCTGGCAGTAGAGGATGTCTCTTG
AGACACCAGTGTCTCCCTCAAGTTCAGGAGGGCCCCCATTAAATTAAGAGAGAGTGGG
AGCAGGAGGCCTGTCTAGATGGCTACTCTTAAGTGGAGTACTGCCCAGAGGGCTGCT
CAGAGTGGGTGGCTGTAACCCTGACAGGGGCTGACAGAGCACACATCCATACTGGTG
AAGGACCTGTGACCCACTGGCCAGACTGGCCGGCCGGAGGAACCCCTAGTGATGGA
GTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCC
CGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAG
AGGGAGTGGCCAA (SEQ ID NO:11)

[0090] In related aspects, the heterologous nucleic acid comprises the following nucleotide sequence or a nucleotide sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical thereto:

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTC
GCCCGACGCCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCTATCGATTGAACCAGGCGCGCCAG
TTAAACAGTCCGGAAGTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCAT
AGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGA
CCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACG
CCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCAC
TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGAC
GGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACT
TGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACG
TTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTAT
TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGCGCGCGCCAG
GCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGG

CAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGG
CGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGGAGTCGCTGCGACGCTGC
CTTCGCCCCGTGCCCCGCTCCGCCGCCGCTCGCGCCGCCCGCCCCGGCTCTGACTG
ACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAAT
TAGCGCTTGGTTTAATGACGGCTTGTTCCTTTTCTGTGGCTGCGTGAAAAGCCTTGAGG
GGCTCCGGGAGGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGT
GTGTGCGTGGGGAGCGCCGCGTGCGGCTCCGCGCTGCCCGGCGGCTGTGAGCGCTG
CGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCG
GGGGCGGTGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCG
GGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCGGTCGGGCTGCAAC
CCCCCTGCACCCCCCTCCCCAGTTGCTGAGCACGGCCCCGGCTTCGGGTGCGGGGC
TCCGTACGGGGCGTGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTG
GGGGTGCCGGGCGGGGCGGGGCCGCCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGC
GCGGCGGCCCCCGAGCGCCGCGGGCTGTGAGGCGCGGCGAGCCGCAGCCATTGC
CTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCG
GAGCCGAAATCTGGGAGGCGCCGCCGACCCCCCTCTAGCGGGCGCGGGGCGAAGCG
GTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCC
GCCGTCCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTC
GGGGGGGACGGGGCAGGGCGGGGTTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGA
GCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTACAGTCTAGAGTCGACGGTC
ACCGGTAGCTAGCACGCCACCATGAGGCTGCTGGCCAAGATCATCTGCCTGATGCT
GTGGGCCATCTGTGTGGCTGAGGACTGTAATGAGCTGCCCCCTAGGAGAAATACAG
AGATCCTGACTGGCTCTTGGTCTGATCAGACCTACCCAGAGGGGCACACAGGCCATCT
ACAAGTGCAGGCCTGGCTATAGATCCCTGGGCAATATCATCATGGTGTGCAGGAAG
GGAGAGTGGGTGGCCCTGAACCCACTGAGAAAGTGCCAGAAGAGGCCATGTGGACA
CCCAGGAGACACACCTTTTGGCACCTTTACACTGACAGGGGGCAATGTGTTTGAGTA
TGGTGTGAAGGCAGTGTATACCTGCAATGAGGGCTACCAGCTGCTGGGAGAGATCA
ACTACAGGGAGTGTGACACAGATGGCTGGACCAATGATATCCCCATCTGTGAGGTG
GTGAAGTGTCTGCCAGTGACTGCCCTGAGAATGGCAAGATAGTGAGCTCTGCCATG
GAGCCTGACAGAGAGTATCACTTTGGCCAGGCTGTGAGGTTTGTGTGCAATAGTGGC
TACAAGATTGAGGGGGATGAGGAGATGCACTGTTTCAGATGATGGCTTCTGGTCTAA
GGAGAAGCCCAAGTGTGTGGAGATCTCCTGTAAGTCTCCTGATGTGATTAATGGCAG
CCCAATCTCCCAGAAGATCATCTATAAGGAGAATGAGAGATTTTCAGTACAAGTGCA
ACATGGGCTATGAGTATTCAGAGAGAGGTGATGCTGTGTGCACAGAGTCTGGATGG
AGGCCCTGCCTAGCTGTGAGGAGAAGTCCACCCTGAAGCCTTGTGACTATCCAGAT
ATCAAGCATGGAGGCCTGTATCATGAGAATATGAGGAGGCCTTACTTCCCAGTGGCT
GTGGGCAAGTACTATTCTACTATTGTGATGAGCACTTTGAGACACCATCTGGCTCT
TACTGGGACCACATCCACTGTACCCAGGATGGCTGGTCTCCTGCTGTGCCTTGCTG
AGAAAGTGTTACTTCCCCTATCTGGAGAATGGCTACAACCAGAATTATGGCAGGAA
GTTTGTGCAGGGCAAGTCCATTGATGTGGCATGCCACCCAGGATATGCACTGCCTAA
GGCACAGACCACAGTGACCTGTATGGAGAATGGCTGGAGCCCAACACCCAGGTGCA
TCAGGGTGAAGACCTGTTCCAAGAGCAGCATTGACATAGAGAATGGCTTCATCTCA
GAGTCCCAGTACACCTATGCCCTGAAGGAGAAGGCCAAGTATCAGTGCAAGCTGGG
ATATGTGACAGCAGATGGAGAAACATCTGGCAGCATCACCTGTGGCAAGGATGGCT
GGTCTGCCCAGCCCACATGCATCAAGAGCATCAAGACTGACTGTCTGTCTCTGCCCA
GCTTTGAGAATGCCATCCCTATGGGTGAGAAGAAGGATGTGTACAAGGCTGGAGAG

CAGGTGACATACACCTGTGCCACATACTATAAGATGGATGGAGCCAGCAATGTGAC
CTGTATCAATTCCAGATGGACAGGCAGACCTACCTGCAGGGATACAAGCTGTGTGA
ACCCACCCACTGTGCAGAATGCCTATATTGTGTCCAGGCAGATGTCTAAGTACCCTT
CTGGAGAGAGAGTGAATATCAGTGCAGATCCCCATATGAGATGTTTGGGGATGAG
GAAGTGATGTGCCTGAATGGCAATTGGACAGAGCCTCCACAGTGCAAGGATAGCAC
TGGCAAGTGTGGACCACCTCCACCAATTGACAATGGAGATATCACATCCTTTCCACT
GTCAGTGTATGCCCCTGCCTCCTCTGTGGAGTATCAGTGCCAGAACCTGTACCAGCT
GGAGGGCAATAAGAGAATCACCTGTAGAAATGGCCAGTGGTCTGAGCCTCCAAAGT
GCCTGCACCCTTGTGTGATCTCCAGAGAGATCATGGAGAACTATAATATAGCCCTGA
GGTGGACAGCCAAGCAGAAGCTGTACTCTAGGACTGGGGAGTCAGTGGAGTTTGTG
TGCAAGAGAGGCTATAGACTGAGCTCCAGAAGCCACACACTGAGGACCACATGCTG
GGATGGCAAGCTGGAGTACCCAACCTGTGCCAAGAGGTGAGCCTAGGACTGTACAG
TACGCGTAACACGTGGGGGATCCAGACATGATAAGATACATTGATGAGTTTGGACA
AACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTAT
TGCTTTATTTGTAAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCAT
TCATTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAA
CCTCTACAAATGTGGTATGGCTGATTATGATCAATGCATGGTCCCAGCCTTCATTGA
GCCTATTGGTCCCCCTCAGAACCCACCCACCTGGCAGTAGAGGATGTCTCTTGAGA
CACCCTGTCTCCCTCAAGTTCAGGAGGGCCCCCATTAAATTAAGAGAGAGTGGGAGC
AGGAGGCCTGTCAGATGGCTACTCTTAAGTGGAGTACTGCCCAGAGGGCTGCTCAG
AGTGGGTGGCTGTAACCCCTGCAGGGGCTGACAGAGCACACATCCATACTGGTGAAG
GACCTGTGACCCACTGGCCAGACTGGCCGCGGCGGAGGAACCCCTAGTGATGGAGTT
GGCCACTCCCTCTCTGCGCTCGCTCGCTCACTGAGGCCGCGCGGCGAAAGCCCGG
GCGTCCGGCGACCTTTGGTCGCGCCCTCAGTGAGCGAGCGAGCGCGCAGAGAGG
GAGTGGCCAA (SEQ ID NO:12)

[0091] In related aspects, the heterologous nucleic acid comprises the following nucleotide sequence or a nucleotide sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical thereto:

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTC
GCCCAGCGCCCGGGCTTTGCCCAGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGA
GGGAGTGGCCAACTCCATCACTAGGGGTTCTATCGATTGAACCAGGCGCGCCAG
TTTAAACAGTCCGGAAGTATTATTAATAGTAATCAATTACGGGGTCATTAGTTCAT
AGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGA
CCGCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACG
CCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCAC
TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGAC
GGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACT
TGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTTCGAGGTGAGCCCCACG
TTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCAATTTTGTATTTATTTAT
TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGGCGCGCGCCAG
GCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGG
CAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGCGGCGG
CGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGGAGTCGCTGCGACGCTGC

CTTCGCCCCGTGCCCCGCTCCGCCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTG
ACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAAT
TAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTGAGG
GGCTCCGGGAGGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGT
GTGTGCGTGGGGAGCGCCGCGTGCGGCTCCGCGCTGCCCGGCGGCTGTGAGCGCTG
CGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCG
GGGGCGGTGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCG
GGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCGGTCGGGCTGCAAC
CCCCCTGCACCCCCCTCCCCAGTTGCTGAGCACGGCCCCGGCTTCGGGTGCGGGGC
TCCGTACGGGGCGTGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTG
GGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGC
GCGGCGGCCCCCGGAGCGCCGCGGCTGTGAGGCGCGGCGAGCCGCAGCCATTGC
CTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCG
GAGCCGAAATCTGGGAGGCGCCGCCGACCCCCCTCTAGCGGGCGCGGGGCGAAGCG
GTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCC
GCCGTCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTC
GGGGGGGACGGGGCAGGGCGGGGTTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGA
GCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTACAGTCTAGAGTCGACGGTC
ACCGGTCAGCTAGCACGCCACCATGAGACTTCTAGCAAAGATTATTTGCCTTATGTT
ATGGGCTATTTGTGTAGCAGAAGATTGCAATGAACTTCCTCCAAGAAGAAATACAG
AAATCTGACAGGTTCTCTGGTCTGACCAAACATATCCAGAAGGCCACCCAGGCTATCT
ATAAATCAGGCCTGGATATAGATCTCTTGAAATATCATAATGGTATGCAAGAAAG
GGAGAATGGGTTGTCTTAATCCATTAAGGAAATGTCAGAAAAGGCCCTGTGGACA
TCCTGGAGATACTCCTTTTGGTACTTTTACCCTTACAGGAGGAAATGTGTTTGAATAT
GGTGTAAGGCTGTGTATACATGTAATGAGGGGTATCAATTGCTAGGTGAGATTAAT
TACAGGGAATGTGACACAGATGGATGGACCAATGATATTCCTATATGTGAAGTTGTG
AAGTGTTTACCAGTGACAGCACAGAGAATGGAAAAATTGTCAGTAGTGCAATGGA
ACCAGATAGAGAATACCATTTTGGACAAGCAGTAAGATTGTATGTAAGTCAAGGCTA
CAAGATTGAAGGAGATGAAGAAATGCATTGTTTCAGATGATGGTTTTTGGAGTAAAG
AGAAACCAAAGTGTGTGGAAATTTTCATGCAATCCCCAGATGTTATAAATGGATCTC
CTATATCTCAGAAGATTATTTATAAGGAGAATGAAAGATTTCATATAAATGTAACA
TGGGTTATGAATACAGTGAAAGAGGAGATGCTGTATGCACTGAATCTGGATGGAGA
CCTTTGCCTTCATGTGAAGAAAAATCAACCTTGAAACCTTGTGATTATCCAGACATT
AAACATGGAGGTCTATATCATGAGAATATGAGAAGACCATACTTTCCAGTAGCTGTA
GGAAAATATTACTCCTATTACTGTGATGAACATTTTGGAGACTCCTTCAGGAAGTTAC
TGGGATCACATTCATTGCACACAAGATGGATGGAGTCCAGCAGTACCATGCCTCAG
AAAATGTTATTTTCTTATTTGGAAAATGGATATAATCAAAATTATGGAAGAAAGTT
TGTACAGGGTAAATCTATAGATGTTGCCTGCCATCCTGGCTATGCTCTTCCAAAAGC
TCAGACCACAGTTACATGTATGGAGAATGGCTGGTCTCCTACTCCCAGATGCATCAG
AGTCAAAACATGTTCCAAATCAAGTATAGATATTGAGAATGGGTTTATTTCTGAATC
TCAGTATACATATGCCTTAAAAGAAAAAGCTAAATATCAATGCAAACCTAGGATATG
TAACAGCAGATGGTGAAACATCAGGATCAATTACATGTGGGAAAGATGGATGGTCA
GCTCAACCCACCTGCATTAAATCTATAAAAAACAGATTGTCTCAGTTTACCTAGCTTT
GAAAATGCCATACCCATGGGAGAGAAGAAGGATGTGTATAAGGCTGGTGAGCAAGT
GACTTACACTTGTGCAACATATTACAAAATGGATGGAGCCAGTAATGTAACATGCAT
TAATAGCAGATGGACAGGAAGGCCAACATGCAGAGACACCTCCTGTGTGAATCCTC

CCACAGTACAAAATGCTTATATAGTGAGTAGACAGATGAGTAAATATCCATCTGGTG
AGAGAGTAAGATATCAATGTAGGAGCCCTTATGAAATGTTTGGGGATGAAGAAGTG
ATGTGTTTAAATGGAACTGGACAGAACCACCTCAATGCAAAGATTCTACAGGAAA
ATGTGGGGCCCCCTCCACCTATTGACAATGGGGACATTACTTCATTCCCCTTGTCAGTA
TATGCTCCAGCTTCATCAGTTGAGTACCAATGCCAGAACTTGTATCAACTTGAGGGT
AACAAGAGGATAACATGTAGAAATGGACAATGGTCAGAACCACCAAAATGCTTACA
TCCCTGTGTAATATCCAGGGAAATTATGGAAAATTATAACATAGCATTAAGGTGGAC
AGCCAAACAGAAGCTTTATTCTAGAACAGGTGAATCAGTTGAATTTGTGTGTAAAAG
AGGATATAGACTTTCATCAAGGTCTCACACATTGAGGACAACATGTTGGGATGGGA
AACTGGAGTATCCAAGTTGTGCAAAAAGATGAGCCTAGGACTGTACAGTACGCGTA
ACACGTGGGGGATCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAA
CTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATT
TGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCAATTTAT
GTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAACCTCTACAA
ATGTGGTATGGCTGATTATGATCAATGCATGGTCCCAGCCTTCATTGAGCCTATTGG
TCCCCCTCAGAACCCACCCACCTGGCAGTAGAGGATGTCTCTTGAGACACCACTGT
CTCCCTCAAGTTCAGGAGGGCCCCCATTAAATTAAGAGAGAGTGGGAGCAGGAGGCCT
GTCAGATGGCTACTCTTAAGTGGAGTACTGCCCAGAGGGCTGCTCAGAGTGGGTGG
CTGTAACCTGCAGGGGCTGACAGAGCACACATCCATACTGGTGAAGGACCTGTGA
CCCCTGGCCAGACTGGCCGGCCGGAGGAACCCCTAGTGATGGAGTTGGCCACTCC
CTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCCGGGCAAAGCCCCGGGCGTCGGGC
GACCTTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCA
A (SEQ ID NO:13)

[0092] In some embodiments, the pharmaceutical composition comprises 1×10^8 to 1×10^{15} vector particles (vp) or vector genomes (vg), 1×10^{10} to 1×10^{13} vector particles or vector genomes, or about 1×10^{10} , about 2×10^{10} , 3×10^{10} , about 4×10^{10} , about 5×10^{10} , about 6×10^{10} , about 7×10^{10} , about 8×10^{10} , about 9×10^{10} , about 1×10^{11} , about 2×10^{11} , about 3×10^{11} , about 4×10^{11} , about 5×10^{11} , about 6×10^{11} , about 7×10^{11} , about 8×10^{11} , about 9×10^{11} , about 1×10^{12} , about 2×10^{12} , about 3×10^{12} , about 4×10^{12} , about 5×10^{12} , about 6×10^{12} , about 7×10^{12} , about 8×10^{12} , about 9×10^{12} or about 1×10^{13} vector particles or vector genomes. In some aspects, the pharmaceutical composition comprises about 1×10^{11} to about 1×10^{12} vector particles or vector genomes.

[0093] In some aspects, the pharmaceutical composition maintains a relative potency in liquid form of at least 90% over a period of at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 3 months, at least 4 months, at least 5 months or at least 6 months at 25°C and 60% relative humidity.

[0094] In exemplary aspects, the composition comprising AAV is storage stable as a liquid for at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, or at least 12 months. In exemplary aspects, greater than 80% of the initial amount of AAV (e.g., the amount of AAV in the composition prior to storage) is potent after the storage period (e.g., a storage period of about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, or about 6 months or longer). In exemplary aspects, greater than 90% of the initial amount of AAV is potent after the storage period (e.g., a storage period of about 1 month, about 2 months, 3 months, about 4 months, about 5 months, or about 6 months or longer). In exemplary aspects, greater than 95% of the initial amount of AAV is potent after the storage period (e.g., a storage period of about 3 months, about 4 months, about 5 months, or about 6 months or longer). In exemplary aspects, the biopotency of the AAV at the end of the storage period is substantially the same as the biopotency of the AAV at the beginning of the storage period. In exemplary aspects, the biopotency of the AAV at the end of the storage period is increased relative to the biopotency of the AAV at the beginning of the storage period. In exemplary aspects, the appearance of the composition at the end of the storage period is substantially the same as the composition at the beginning of the storage period. In exemplary aspects, the appearance of the composition at the end of the storage period is characterized by having no visible particles. In exemplary aspects, the particle concentration of the composition at the end of the storage period is substantially the same as the particle concentration of the composition at the beginning of the storage period. In exemplary aspects, the particle concentration of the composition at the end of the storage period is determined by microflow imaging (MFI).

[0095] In some embodiments, the pharmaceutical composition is maintained at about -60°C or below for a substantial portion (e.g., the duration) of the storage period. In other embodiments, the pharmaceutical composition is maintained at from about 2°C to about 8°C for a substantial portion (e.g., the duration) of the storage period.

[0096] In some embodiments, a method for treating a disorder in a subject in need thereof is provided, the method comprising administering to the subject a pharmaceutical composition as described herein in an amount effective to treat the disorder. In some preferred embodiments,

the pharmaceutical composition is administered intraocularly to a human with a VEGF-related ocular disorder in an amount effective to treat the VEGF-related ocular disorder, preferably wherein the pharmaceutical composition is administered via intravitreal, subretinal and/or suprachoroidal injection, more preferably via a single intravitreal injection. In other embodiments, the disorder is selected from those listed at paragraphs 218 and 246 of US Patent Application Publication No. 2020/0282077.

[0097] The formulations disclosed herein may be formulated for administration via known methods, such as intraocular administration (e.g., via intravitreal, subretinal and/or suprachoroidal administration). In some preferred embodiments, the pharmaceutical composition is administered intraocularly to a human, preferably wherein the pharmaceutical composition is administered via intravitreal, subretinal and/or suprachoroidal injection, more preferably via a single intravitreal injection.

[0098] In some embodiments, the treatment regimen includes administering one or more doses over an extended period of time. In certain cases, a single dose (e.g., a single dosage unit) is administered to the subject, and the initial dose may be followed by one or more doses administered to the subject at a subsequent time. In some instances, more than one dose (e.g., more than one dosage unit) is administered to the subject, and the initial doses may be followed by one or more doses administered to the subject at a subsequent time. For example, a single dose (e.g., a single dosage unit) may be administered to the subject, and the single dose may be followed by a single dose administered to the subject at a subsequent time. Additional single doses may be administered at subsequent points in time. In other cases, a single dose (e.g., a single dosage unit) may be administered to the subject, and the single dose may be followed by two doses administered to the subject at a subsequent time. Additional single or multiple doses may be administered at subsequent points in time.

[0099] In certain embodiments, dosage units of the present disclosure can be administered prior to, concurrent with, or subsequent to other active agents for treating related or unrelated conditions, e.g., in combination therapy. Administration of separate pharmaceutical compositions can be performed simultaneously or at different times (e.g., sequentially, in either order, on the same day, or on different days), as long as a therapeutically effective effect of the combination of

these substances is caused in the subject undergoing therapy. Accordingly, aspects of the present disclosure further include combination therapies. In certain embodiments, the subject method includes administering a therapeutically effective amount of one or more additional active agents. By combination therapy is meant that a AAV composition (e.g., as described herein) can be used in a combination with another therapeutic agent to treat a single disease or condition. In certain embodiments, a compound of the present disclosure is administered concurrently with the administration of another therapeutic agent, which can be administered as a component of a composition including the compound of the present disclosure or as a component of a different composition. In certain embodiments, a composition including a compound of the present disclosure is administered prior or subsequent to administration of another therapeutic agent.

EXAMPLES

[00100] The following examples illustrate preferred embodiments of the present invention and are not intended to limit the scope of the invention in any way. While this invention has been described in relation to its preferred embodiments, various modifications thereof will be apparent to one skilled in the art from reading this application.

Example 1

[00101] The below sections summarize key findings that led to identification of an improved rAAV formulation (10 mM Tris buffer, pH 7.9, 180 mM NaCl, 0.005% Pluronic F68) which exhibits reduced risk of subvisible particle formation as well as improved liquid storage stability, which is consistent with the stabilization provided by increased ionic strength and reduced rAAV surface adsorption by use of surfactant and the importance of optimizing formulation pH. Wright, JF et al. Mol Ther. 2005 Jul;12(1):171-8); Patricio MI, Mol Ther Methods Clin Dev. 2019 Nov 20;17:99-106; Srivastava, A. et al. Journal of Pharmaceutical Sciences, Volume 110, Issue 7, 2021. The trend of increasing stability of rAAV (comprising a capsid protein of SEQ ID NO:3 and a nucleic acid comprising the nucleotide sequence of SEQ ID NO:6), at higher pH initially drove the selection of the Tris buffer component (Tris Hydrochloride/Tromethamine) in the improved rAAV formulation. However, in at least one study Tris buffer was also shown to reduce susceptibility to chemical degradation (e.g., deamidation and oxidation) compared to

Hepes and Phosphate formulations made at the same target pH and equivalent concentrations of sodium chloride and Pluronic F68.

[00102] Table 1. Improved rAAV Formulation Components and Functions

Component	Function	Target Concentration
Pluronic® F68	Surfactant	0.005%
Tris Hydrochloride	Buffering Agent	6.45 mM
Tromethamine	Buffering Agent	3.55 mM
Sodium Chloride	Tonicity	180 mM
Water for Injection	Solvent	QS

[00103] rAAV, comprising a capsid protein of SEQ ID NO:3 and a nucleic acid comprising the nucleotide sequence of SEQ ID NO:6, was buffer-exchanged into 5 different buffers (Form 1-5) and subjected to various forced degradation conditions. A summary of titer, DLS and subvisible particle testing results can be found in Table 2. Titers were similar for most conditions, except after 3-day storage at 37°C. Interestingly, 37°C titer values were suggestive of increased thermal stability for Form 4 (10 mM Histidine, pH 6, 180 mM NaCl, 0.005% F68). However, Form 4 showed markedly decreased colloidal stability (i.e., increased poly-dispersity index (PDI) and decreased % area, 10-100 d.nm, via dynamic light scattering (DLS) and increased SVPs) at most conditions tested. Conversely, Form 2 (10 mM Sodium Phosphate, pH 7.0, 170 mM NaCl, 0.005% F68), Form 3 (10 mM Sodium Phosphate, pH 7.0, 180 mM NaCl, 0.005% F68) and Form 5 (10 mM Tris buffer, pH 8.0, 180 mM NaCl, 0.005% F68) showed improved colloidal stability, relative to the Form 1 control (DPBS, 0.005% F68), at most conditions. Representative backgrounded membrane images (Figure 1) have been included for the 3-day storage condition to further illustrate the higher risk of subvisible particle formation exhibited by Form 1 and Form 4. These results suggested that pH 7 or pH 8 formulations containing increased sodium chloride were likely to result in an improvement over buffer (DPBS, 0.005% F68).

[00104] Table 2. Titer, DLS, and SVP Testing Results

Form	Description	Condition	Titer [vg/ml] ¹	PDI ²	% Area (10 - 100 nm) ³	Subvisible Particle Testing Results ⁴			
						Particles/ml			
						≥2μm	≥10μm	≥25μm	≥50μm
1	DPBS	T=0	2.53E+13	0.054	100.0	6120	6	0	0
2	10 mM Na Phosphate, pH 7, 170 mM NaCl	T=0	2.17E+13	0.012	100.0	280	13	0	0
3	10 mM Na Phosphate, pH 7, 180 mM NaCl	T=0	2.27E+13	0.019	100.0	240	13	0	0
4	10 mM Histidine, pH 6, 180 mM NaCl	T=0	2.43E+13	0.343	98.0	253	0	0	0
5	10 mM Tris, pH 8, 180 mM NaCl	T=0	2.14E+13	0.020	100.0	360	27	0	0
1	DPBS	3XFT	2.51E+13	0.123	100.0	9720	67	40	13
2	10 mM Na Phosphate, pH 7, 170 mM NaCl	3XFT	2.11E+13	0.015	100.0	453	27	0	0
3	10 mM Na Phosphate, pH 7, 180 mM NaCl	3XFT	2.12E+13	0.009	100.0	480	53	0	0
4	10 mM Histidine, pH 6, 180 mM NaCl	3XFT	2.29E+13	0.714	93.2	7267	40	13	13
5	10 mM Tris, pH 8, 180 mM NaCl	3XFT	2.25E+13	0.025	100.0	333	27	0	0
1	DPBS	3dy @ 37C	2.21E+13	0.092	100.0	6400	1227	237	0
2	10 mM Na Phosphate, pH 7, 170 mM NaCl	3dy @ 37C	1.91E+13	0.013	100.0	2280	107	13	0
3	10 mM Na Phosphate, pH 7, 180 mM NaCl	3dy @ 37C	2.08E+13	0.021	100.0	1347	40	13	0
4	10 mM Histidine, pH 6, 180 mM NaCl	3dy @ 37C	2.40E+13	0.332	98.9	132040	30120	3040	267
5	10 mM Tris, pH 8, 180 mM NaCl	3dy @ 37C	1.70E+13	0.020	100.0	1387	67	13	13
1	DPBS	10XFT	2.55E+13	0.138	100.0	357373	9440	267	0
2	10 mM Na Phosphate, pH 7, 170 mM NaCl	10XFT	2.14E+13	0.012	100.0	1280	67	27	27
3	10 mM Na Phosphate, pH 7, 180 mM NaCl	10XFT	2.16E+13	0.014	100.0	1213	80	40	27
4	10 mM Histidine, pH 6, 180 mM NaCl	10XFT	2.40E+13	1.368	80.8	1624680	16987	93	27
5	10 mM Tris, pH 8, 180 mM NaCl	10XFT	2.09E+13	0.008	100.0	6747	213	40	0
1	DPBS	1M @ RT	2.17E+13	0.013	100.0	294560	340	100	40
2	10 mM Na Phosphate, pH 7, 170 mM NaCl	1M @ RT	2.04E+13	0.004	100.0	173	27	13	13
3	10 mM Na Phosphate, pH 7, 180 mM NaCl	1M @ RT	2.04E+13	0.017	100.0	253	40	13	13
4	10 mM Histidine, pH 6, 180 mM NaCl	1M @ RT	2.30E+13	0.215	99.7	190213	15493	120	27
5	10 mM Tris, pH 8, 180 mM NaCl	1M @ RT	2.03E+13	0.033	100.0	730	190	20	0

All formulations also contain 0.005% Pluronic F58

¹ T=0 values for Form 1 and Form 2 represent n=2 averages. All other titer values are n=1² Polydispersity Index³ % Area by Volume⁴ Mean counts for n=3 wells except for Form 1 and Form 5 after 1M RT storage (n=2 wells)

[00105] pH Optimization

[00106] The next studies aimed to further assess pH dependence. In the first study, rAAV (comprising a capsid protein of SEQ ID NO:3 and a nucleic acid comprising the nucleotide sequence of SEQ ID NO:6) was buffer-exchanged into 6 different formulations containing Sodium Phosphate buffer ranging from pH 6.0 to 7.0. In the second study, rAAV (comprising a capsid protein of SEQ ID NO:3 and a nucleic acid comprising the nucleotide sequence of SEQ ID NO:6) was buffer-exchanged into Phosphate or Tris buffer at a pH 7.0, 7.4 and 7.8. These studies (along with subsequent Formulation Development experiments) included testing for aflibercept (AFLIB) expression using a modified form of the release assay tailored to high throughput sample analysis. Aflibercept expression was reported as relative potency (% RP), or AFLIB expression relative to a standard. Like prior results, the first of these studies demonstrated decreased colloidal stability when pH was decreased from 7.0 to 6.0 (Table 3). Decreased relative potency (Figure 2) was also observed for lower pH formulations in response

to thermal stress. Conversely, a decreased risk of subvisible particle formation and equal or better 2-week storage stability at 25°C/60%RH was noted for the rAAV formulated in 10 mM Sodium Phosphate, pH 7.0, 175 mM NaCl, 0.005% F68 compared to the rAAV formulated in Form 1 (DPBS, 0.005% F68). The results of the second study (Table 4) demonstrated a further reduction in subvisible particle risk as pH was increased from 7.0 to 7.8. Most interesting was rAAV formulated in 10 mM Tris buffer, pH 7.8, 180 mM NaCl, 0.005% F68 which not only showed low subvisible particle counts for all conditions but also the highest relative potency (98.2%) after 2-week storage at 25°C/60%RH (Figure 3).

[00107] Table 3 Study Results

Form	Description	Condition	pH	Filter (µg/ml) ¹	Relative Potency %	Polydispersity Index ²	% Area by Volume ³	% Area by Intensity ²	Replicate Weils	Subvisible Particle Testing Results				
										Particles/ml				
										2µm-10µm	≥10µm	≥25µm	≥50µm	
1	DPBS	T=0	7.00	2.08E+13	100.7%	0.087	100.0	100.0	2	43900	60	0	0	
2	10 mM Na Phosphate, pH 6.0, 175 mM NaCl	T=0	5.98	2.05E+13	91.9%	0.171	99.9	97.9	3	2027	0	0	0	
3	10 mM Na Phosphate, pH 6.2, 175 mM NaCl	T=0	6.23	1.70E+13	Not Determined	0.124	99.9	98.4	1	1720	40	0	0	
4	10 mM Na Phosphate, pH 6.3, 175 mM NaCl	T=0	6.40	2.08E+13	79.5%	0.100	100.0	100.0	2	27440	60	0	0	
5	10 mM Na Phosphate, pH 6.8, 175 mM NaCl	T=0	6.87	1.92E+13	84.1%	0.045	100.0	100.0	3	587	13	0	0	
6	10 mM Na Phosphate, pH 7.0, 175 mM NaCl	T=0	7.02	1.85E+13	97.8%	0.018	100.0	100.0	2	420	40	26	0	
1	DPBS	3XFT	7.00	2.04E+13	103.4%	0.094	100.0	100.0	2	164810	20	0	0	
2	10 mM Na Phosphate, pH 6.0, 175 mM NaCl	3XFT	6.12	2.08E+13	35.9%	0.299	98.9	83.6	3	145575	27	27	0	
3	10 mM Na Phosphate, pH 6.2, 175 mM NaCl	3XFT	6.22	1.73E+13	Not Determined	0.392	97.9	79.8	3	25032	27	0	0	
4	10 mM Na Phosphate, pH 6.3, 175 mM NaCl	3XFT	6.60	1.85E+13	81.0%	0.165	99.9	93.0	2	16760	30	0	0	
5	10 mM Na Phosphate, pH 6.8, 175 mM NaCl	3XFT	6.87	1.90E+13	103.9%	0.057	100.0	100.0	2	440	40	0	0	
6	10 mM Na Phosphate, pH 7.0, 175 mM NaCl	3XFT	7.11	1.79E+13	104.6%	0.025	100.0	100.0	2	560	40	26	0	
1	DPBS	T=0.5M @ 25C/60%RH	7.00	2.02E+13	65.4%	0.009	100.0	100.0	3	85467	11453	53	0	
2	10 mM Na Phosphate, pH 6.0, 175 mM NaCl	T=0.5M @ 25C/60%RH	6.13	2.14E+13	38.3%	0.040	100.0	100.0	3	55427	153	0	0	
3	10 mM Na Phosphate, pH 6.2, 175 mM NaCl	T=0.5M @ 25C/60%RH	6.39	1.95E+13	Not Determined	0.051	100.0	100.0	3	114590	1253	0	0	
4	10 mM Na Phosphate, pH 6.3, 175 mM NaCl	T=0.5M @ 25C/60%RH	6.50	2.45E+13	48.5%	0.143	99.9	99.1	3	400	0	0	0	
5	10 mM Na Phosphate, pH 6.8, 175 mM NaCl	T=0.5M @ 25C/60%RH	6.90	2.48E+13	55.9%	0.051	100.0	100.0	3	8373	63	13	0	
6	10 mM Na Phosphate, pH 7.0, 175 mM NaCl	T=0.5M @ 25C/60%RH	7.09	2.49E+13	55.0%	0.024	100.0	100.0	3	560	13	0	0	
1	DPBS	T=1M @ 25C/60%RH	7.12	1.93E+13	61.6%	0.047	Not Determined	100.0	3	79387	7267	40	27	
2	10 mM Na Phosphate, pH 6.0, 175 mM NaCl	T=1M @ 25C/60%RH	6.19	1.73E+13	20.6%	0.521	Not Determined	92.0	3	144567	280	40	0	
3	10 mM Na Phosphate, pH 6.2, 175 mM NaCl	T=1M @ 25C/60%RH	6.34	1.76E+13	Not Determined	0.418	Not Determined	90.1	3	150640	5507	40	13	
4	10 mM Na Phosphate, pH 6.3, 175 mM NaCl	T=1M @ 25C/60%RH	6.65	1.94E+13	34.9%	0.251	Not Determined	93.9	3	480	27	0	0	
5	10 mM Na Phosphate, pH 6.8, 175 mM NaCl	T=1M @ 25C/60%RH	6.89	1.89E+13	57.5%	0.087	Not Determined	100.0	3	5773	147	27	0	
6	10 mM Na Phosphate, pH 7.0, 175 mM NaCl	T=1M @ 25C/60%RH	7.04	2.04E+13	41.4%	0.015	Not Determined	100.0	3	1080	53	27	13	

All formulations also contain 0.005% Pluronic F68

¹ T=0.5M @ 25C/60%RH values for Form 4 and Form 5 represent n=2 averages. All other filter values are n=1

² Polydispersity index, T=1M @ 25C/60%RH data collected on the Wyatt DynaPro plate reader. All other data collected on the Malvern Zetasizer ultra.

³ % Area [10-100 d.nm], T=1M @ 25C/60%RH data collected on the Wyatt DynaPro plate reader. All other data collected on the Malvern Zetasizer Ultra.

[00108] Table 4. Study Results

								Subvisible Particle Testing Results				
Form	Description	Condition	pH	Titer (vg/ml)	Relative Potency %	Polydispersity Index	% Area by Intensity (10-100 d.nm)	Replicate Wells	Particles/ml			
									2µm-10µm	≥10µm	≥25µm	≥50µm
1	10 mM Phosphate, pH 7.0, 180 mM NaCl	T=0	7.11	7.13E+12	119.5%	0.057	100.0	3	147	0	0	0
2	10 mM Phosphate, pH 7.4, 180 mM NaCl	T=0	7.42	6.65E+12	108.0%	0.088	96.7	3	187	6	6	0
3	10 mM Phosphate, pH 7.8, 180 mM NaCl	T=0	7.84	7.18E+12	120.2%	0.017	100.0	3	153	27	27	0
4	10 mM Tris, pH 7.0, 180 mM NaCl	T=0	7.05	7.21E+12	108.4%	0.182	95.7	3	320	13	13	0
5	10 mM Tris, pH 7.4, 180 mM NaCl	T=0	7.36	6.95E+12	116.1%	0.632	100.0	2	280	6	6	0
6	10 mM Tris, pH 7.8, 180 mM NaCl	T=0	7.74	7.12E+12	107.5%	0.031	100.0	2	220	20	0	0
1	10 mM Phosphate, pH 7.0, 180 mM NaCl	3XFT	7.12	6.70E+12	89.6%	0.067	98.1	3	387	267	30	0
2	10 mM Phosphate, pH 7.4, 180 mM NaCl	3XFT	7.42	6.61E+12	90.1%	0.100	98.0	3	1133	52	13	13
3	10 mM Phosphate, pH 7.8, 180 mM NaCl	3XFT	7.88	7.07E+12	99.1%	0.017	100.0	2	140	0	0	0
4	10 mM Tris, pH 7.0, 180 mM NaCl	3XFT	6.97	6.95E+12	21.8%	0.178	95.2	3	133	6	6	0
5	10 mM Tris, pH 7.4, 180 mM NaCl	3XFT	7.39	6.95E+12	96.5%	0.055	100.0	2	100	6	0	0
6	10 mM Tris, pH 7.8, 180 mM NaCl	3XFT	7.73	6.80E+12	97.0%	0.032	100.0	2	380	0	0	0
1	10 mM Phosphate, pH 7.0, 180 mM NaCl	T=2wk @ 25C/60%RH	7.08	6.92E+12	86.1%	0.052	100.0	3	3940	1000	240	67
2	10 mM Phosphate, pH 7.4, 180 mM NaCl	T=2wk @ 25C/60%RH	7.30	7.12E+12	80.6%	0.072	100.0	3	470	46	13	0
3	10 mM Phosphate, pH 7.8, 180 mM NaCl	T=2wk @ 25C/60%RH	7.63	6.88E+12	86.4%	0.01E	100.0	3	440	27	0	0
4	10 mM Tris, pH 7.0, 180 mM NaCl	T=2wk @ 25C/60%RH	6.98	6.69E+12	66.7%	0.322	92.8	3	827	27	13	0
5	10 mM Tris, pH 7.4, 180 mM NaCl	T=2wk @ 25C/60%RH	7.35	6.55E+12	90.8%	0.130	97.2	3	467	27	0	0
6	10 mM Tris, pH 7.8, 180 mM NaCl	T=2wk @ 25C/60%RH	7.75	6.94E+12	98.2%	0.082	98.8	3	387	13	0	0

All formulations also contain 0.005% Pluronic F68

[00109] The results of the preceding study suggested that formulating an rAAV (e.g., comprising a capsid protein of SEQ ID NO:3) in 10 mM Tris buffer, pH 7.8, 180 mM NaCl, 0.005% F68 would not only meet the primary objective (i.e., reduced subvisible particles) but might also result in improved liquid storage stability. Therefore, pH 7.8 was revisited (in Phosphate, Tris, and Hepes) and compared against Form 1 (DPBS, 0.005% F68) during storage at 25°C/60%RH. Relative potency results can be found in Table 5. All four formulations behaved very similarly, possibly with subtle improvements associated with pH 7.8 formulations (Form 1-3) after 2-week storage @ 25°C/60%RH. Additionally, mass spectrometry results (Table 6) revealed no new degradants for the pH 7.8 formulations at T=0. However, Phosphate and Hepes pH 7.8 formulations did appear to show an increased rate of deamidation relative to Form 1 during 2-week storage @ 25°C/60%RH. Conversely, the Tris, pH 7.8 formulation showed a deamidation profile that was very similar to Form 1 while also exhibiting a reduced risk of oxidation.

[00110] Table 5. Relative Potency (% RP) Summary

Form	Description	Condition	pH	Titer (vg/mL)	Relative Potency % ¹	Relative Potency % ²
1	10 mM Phosphate, pH 7.8, 180 mM NaCl	T=0	7.65	1.78E+12	120.0%	98.4%
2	10 mM Tris, pH 7.8, 180 mM NaCl	T=0	7.84	1.79E+12	121.4%	99.5%
3	Hepes, pH 7.8, 180 mM NaCl	T=0	7.87	1.69E+12	122.0%	105.6%
4	DPBS	T=0	6.97	1.75E+12	115.0%	100.0%
1	10 mM Phosphate, pH 7.8, 180 mM NaCl	T=2wk @ 25C/60%RH	7.63	1.57E+12	89.1%	80.8%
2	10 mM Tris, pH 7.8, 180 mM NaCl	T=2wk @ 25C/60%RH	7.81	1.44E+12	90.5%	77.6%
3	Hepes, pH 7.8, 180 mM NaCl	T=2wk @ 25C/60%RH	7.78	1.59E+12	93.5%	76.6%
4	DPBS	T=2wk @ 25C/60%RH	7.01	1.56E+12	85.8%	72.5%
1	10 mM Phosphate, pH 7.8, 180 mM NaCl	T=1M @ 25C/60%RH	7.67	1.92E+12	83.4%	59.4%
2	10 mM Tris, pH 7.8, 180 mM NaCl	T=1M @ 25C/60%RH	7.86	1.70E+12	79.3%	65.5%
3	Hepes, pH 7.8, 180 mM NaCl	T=1M @ 25C/60%RH	7.83	1.75E+12	78.6%	66.8%
4	DPBS	T=1M @ 25C/60%RH	7.06	1.81E+12	76.1%	65.4%

All formulations also contain 0.005% Pluronic F68

¹ N=3 (From Plates 1-3), calculated relative to 4D-150 reference standard

² N=1 (From Plate 4), calculated relative to Form 4, T=0

[00111] Table 6. Mass Spectrometry Results

Sites	Start AA	Sequence	Modifications	Form 1		Form 2		Form 3		Form 4	
				10 mM Phosphate, pH 7.8, 180 mM NaCl, 0.005% F68		10 mM Tris, pH 7.8, 180 mM NaCl, 0.005% F68		10 mM Hepes, pH 7.8, 180 mM NaCl, 0.005% F68		DPBS, 0.005% F68	
				T = 0	T=2wk @ 25C/60%RH	T = 0	T=2wk @ 25C/60%RH	T = 0	T=2wk @ 25C/60%RH	T = 0	T=2wk @ 25C/60%RH
R67n	52	(K)PLGPHGLDR(G)	n-Deamidated	4.10%	9.50%	1.70%	5.80%	3.60%	8.40%	0.00%	4.10%
N228n	219	(A)DGVGSGGNWHD(G)	n-Deamidated	2.80%	2.80%	0.70%	0.70%	1.30%	3.00%	1.10%	1.90%
N253 or 254n	246	(R)TWALPTYnHLVY(G)	n-Deamidated	1.40%	1.50%	1.80%	1.80%	2.20%	3.00%	1.00%	1.70%
N475n	475	(R)WLFSPCVPL(G)	n-Deamidated	0.60%	1.10%	0.30%	0.20%	0.50%	1.10%	0.60%	0.60%
N495 or 496 or 497n	491	(K)TSAGnLnSEKSWTSATK(Y)	n-Deamidated	4.10%	7.10%	2.00%	8.50%	2.80%	4.50%	2.30%	4.20%
N498n	495	(R)LNAPLDQYLVLSP(E)	m-Oxidized methionine	4.20%	4.40%	1.30%	1.20%	9.00%	5.60%	2.40%	4.50%
N558n	557	(K)NVVFDEEELRTI	m-Oxidized methionine	0.90%	1.10%	0.40%	0.40%	0.60%	1.30%	0.60%	0.90%
N614n	599	(R)QAATAGVNTQGLPGnVWQGR(D)	m-Oxidized methionine	1.40%	2.30%	0.70%	0.70%	1.50%	2.50%	1.10%	1.70%
S149n	143	(K)KRPVPH-PVFFPRNCTSK(A)	s-phosphorylated	9.70%	9.70%	9.40%	8.10%	9.50%	9.50%	9.80%	9.70%
Total			Deamidation	13.10%	21.20%	7.50%	11.60%	10.30%	20.10%	8.60%	12.50%
			Oxidation	6.50%	7.60%	2.40%	2.90%	5.20%	9.40%	4.10%	7.10%
			Phosphorylation	9.70%	9.70%	9.40%	8.10%	9.50%	9.50%	9.80%	9.70%

[00112] Confirmatory Forced Degradation Studies

[00113] Collectively, subvisible particle data, relative potency and chemical stability all suggested that a Tris based buffer would make a suitable choice for an improved rAAV formulation. Therefore, the intent of the next formulation development study was to perform a

bracketed (Tris, pH 7.8 ± 0.3) forced degradation experiment in a representative container closure system (i.e., 0.3 mL fill in a 2 mL CZ vial). Samples were tested at T=0, after 3 freeze-thaw cycles (3XFT), and after 2-week storage at 2-8°C and 25°C/60%RH. Results can be found in Table 8. The FDA requires “GT vector-based final products should be tested for particulate matter, and the test method and release criteria should follow USP <789>.” See <https://www.fda.gov/media/124641/download> Subvisible particle testing yielded results that met USP <789> limits for 11 of 12 tested samples. Additionally, 100% area (10-100 d.nm. by Volume) was observed for 11 of 12 tested samples. These two results appeared to confirm increased physical stability within the range tested. Relative potency also proved to be very similar for all three formulations. Each showed a reduction in aflibercept expression after 2 week storage at 25°C/60%RH and ≥ 100% relative potency after 2 week storage at 2-8°C.

[00114] Table 7. USP <789> Subvisible Particle Test Limits

	Diameter		
	≥ 10 μm	≥ 25 μm	≥ 50 μm
Number of Particles	50/mL	5/mL	2/mL

[00115] Table 8. Results Summary

Form	Description	Condition	pH ¹	Titer {vg/ml}	Relative Potency %	Polydispersity index	% Area by Volume {10-100 d.nm}	Subvisible Particle Testing Results				
								Replicate Wells	Particles/ml			
									≥2μm	≥10μm	≥25μm	≥50μm
1	10 mM Tris, pH 7.8, 180 mM NaCl	T=0	7.78	1.52E+12	113.9%	0.123	100.0	3	433	17	17	0
2	10 mM Tris, pH 8.0, 180 mM NaCl	T=0	7.98	2.07E+12	112.3%	0.078	100.0	3	333	0	0	0
3	10 mM Tris, pH 8.3, 180 mM NaCl	T=0	8.30	1.45E+12	109.3%	0.192	99.2	3	267	0	0	0
1	10 mM Tris, pH 7.8, 180 mM NaCl	3XFT	Not Tested	1.35E+12	Not Tested	0.018	100.0	3	1100	17	0	0
2	10 mM Tris, pH 8.0, 180 mM NaCl	3XFT	Not Tested	1.99E+12	Not Tested	0.105	100.0	3	723	0	0	0
3	10 mM Tris, pH 8.3, 180 mM NaCl	3XFT	Not Tested	1.57E+12	Not Tested	0.036	100.0	3	467	0	0	0
1	10 mM Tris, pH 7.8, 180 mM NaCl	T=2wk @ 2-8°C	Not Tested	1.52E+12	101.4%	0.018	100.0	3	463	0	0	0
2	10 mM Tris, pH 8.0, 180 mM NaCl	T=2wk @ 2-8°C	Not Tested	2.02E+12	102.6%	0.011	100.0	3	317	0	0	0
3	10 mM Tris, pH 8.3, 180 mM NaCl	T=2wk @ 2-8°C	Not Tested	1.47E+12	100.8%	0.012	100.0	3	250	0	0	0
1	10 mM Tris, pH 7.8, 180 mM NaCl	T=2wk @ 25°C/60%RH	Not Tested	1.47E+12	86.0%	0.042	100.0	3	400	0	0	0
2	10 mM Tris, pH 8.0, 180 mM NaCl	T=2wk @ 25°C/60%RH	Not Tested	1.94E+12	78.3%	0.020	100.0	3	867	33	0	0
3	10 mM Tris, pH 8.3, 180 mM NaCl	T=2wk @ 25°C/60%RH	Not Tested	1.38E+12	83.7%	0.012	100.0	3	467	0	0	0

All formulations also contain 0.005% Pluronic F68

¹ pH testing was performed on a separate vial that was treated similar to T=0. Also noting that sample pH was measured after room temperature equilibration (~20.0°C)

[00116] This confirmatory development study results ultimately led to the nomination of the formulation which was initially described as 10 mM Tris buffer, pH 8.0, 180 mM NaCl, 0.005% F68. However, it was noted that most pH testing conducted during formulation development was measured at room temperature (typically ranging from ~ 19-21°C). Therefore, in an effort to improve the robustness of the diluent manufacture (specifically the known temperature

dependence exhibited by Tris buffer) it was decided that all future pH testing (in-process and at release) should be conducted at 25°C in accordance with USP <791>. Therefore, three preparations of the lead formulation buffer were prepared, and pH tested at 20°C and 25°C (Table 8). As expected, pH measurement at 25°C yielded a drop in pH of approximately 0.1 pH unit. This prompted a corresponding change in target pH (7.9 ± 0.3) as well as buffer description (10 mM Tris buffer, pH 7.9, 180 mM NaCl, 0.005% F68).

[00117] Table 8. Lead Formulation Buffer pH Measured at 20°C and 25°C

	pH	Temperature (C°)	pH	Temperature (C°)
Prep 1	8.01	20.0	7.87	24.9
Prep 2	8.01	20.0	7.87	25.1
Prep 3	8.01	19.9	7.86	25.1
Average	8.01	20.0	7.87	25.0
Std DEV	0.000	0.06	0.006	0.113
% RSD	0.0%	0.3%	0.1%	0.5%

Example 2

[00118] The impact of sodium chloride concentration (140-200 mM) was evaluated in the below formulations at T=0 and after 3 freeze-thaw cycles (3XFT). All three formulations contained rAAV, comprising a capsid protein of SEQ ID NO:3 and a nucleic acid comprising the nucleotide sequence of SEQ ID NO:6, at a target concentration of $\sim 1.6 \times 10^{12}$ vg/mL

20 mM Tris, pH 8¹, 140 mM NaCl, 0.005% F68

20 mM Tris, pH 8¹, 180 mM NaCl, 0.005% F68

20 mM Tris, pH 8¹, 200 mM NaCl, 0.005% F68

¹ pH 8 is the target pH at room temperature. This is expected to be approximately 0.1 pH unit lower (i.e., 7.9) when measured at 25°C

[00119] Sample testing on a Wyatt DynaPro III DLS Plate Reader (Figure 4) demonstrated similar colloidal stability, i.e., > 90% Area (10-100 d.nm) by Intensity, across the range of sodium chloride evaluated.

[00120] Samples were, subsequently, 0.45 μ m filtered, spiked with 20X Sybr Gold and tested by Differential Scanning Fluorimetry (Uncle Instrument by Unchained Labs). Final Fluorescence (Final FL) values reported in Figure 5 represent the fluorescent emission signal ranging from 500-650 nm collected at 15°C, after samples were subjected to a thermal melt (15-95°C). Results suggest similar amounts of accessible ssDNA (i.e., relative titer) present in all three formulations at T=0. 3XFT samples showed slightly higher signal recoveries for sodium chloride concentrations $\geq$ 180 mM NaCl. However, none of the filtered, 3XFT stressed samples showed a recovery of less than 78% (Figure 6).

[00121] Many of the study results above contain pH values which were measured at room temperature. However, as described in Table 8, Tris buffer exhibits temperature dependence. This temperature dependence has also been captured in a separate study in which a Tris, pH 7.8 buffer was measured at room temperature (the same temperature it was initially made and tested at) and after equilibration in either a 2-8°C or a 25°C/60%RH stability chamber. The results indicate that a pH shift of approximately -0.1 pH unit should be expected for a 10 mM Tris buffer prepared at room temperature and then stored at 25°C (consistent with Table 8). Additionally, a 0.3 pH unit shift is expected for 10 mM Tris buffer prepared at room temperature and then stored at 2-8°C. This, in turn, implies that rAAV comprising a capsid protein of SEQ ID NO:3 has shown stability in Tris buffered saline solutions ranging from approximately pH 7.3 (pH 7.4 formulation stored at 25C/60%RH) to 8.6 (pH 8.3 formulation stored at 2-8C) as shown in Table 9 below.

[00122] Table 9. Tris Buffer Temperature Dependence

Buffer	2-8°C		Room Temp.	25°C/60%RH	
	pH	Δ pH ¹	pH	pH	Δ pH ¹
10 mM Tris, pH 7.8, 180 mM NaCl, 0.005% F68	8.179	0.323	7.856	7.751	-0.105

¹ change in pH relative to pH at room temperature

Example 3

[00123] The long-term stability of rAAV, comprising a capsid protein of SEQ ID NO:3 and a nucleic acid comprising the nucleotide sequence of SEQ ID NO:6, formulated in 10 mM Tris,

180 mM NaCl, 0.005% Pluronic F68 has, subsequently, been evaluated at both $\leq -60^{\circ}\text{C}$ and $2-8^{\circ}\text{C}$.

[00124] In short, product development lots 4DER000096 and 4DER000097 (2-mL CZ vial filled with rAAV, comprising a capsid protein of SEQ ID NO:3 and a nucleic acid comprising the nucleotide sequence of SEQ ID NO:6, formulated in 10 mM Tris, 180 mM NaCl, 0.005% Pluronic F68), were evaluated in an abbreviated long-term stability study conducted at $\leq -60^{\circ}\text{C}$ and $2-8^{\circ}\text{C}$. pH, subvisible particles, genomic titer, transgene expression (protein-based) and monodispersion results can be found in Table 10 and Table 11 below. Results for clinical lot 4D2210031 can be found in Table 12.

[00125] Table 10: Stability at $\leq -60^{\circ}\text{C}$ and $2-8^{\circ}\text{C}$

Storage at $\leq -60^{\circ}\text{C}$		Test Interval (Months)		
Attribute	Test Method	0	3	12
pH	Potentiometry	7.6	7.5	7.5
Subvisible Particles	Background Membrane Imaging (Particles/mL)	$\geq 10\ \mu\text{m}: 0$	$\geq 10\ \mu\text{m}: 0$	$\geq 10\ \mu\text{m}: 0$
		$\geq 25\ \mu\text{m}: 0$	$\geq 25\ \mu\text{m}: 0$	$\geq 25\ \mu\text{m}: 0$
		$\geq 50\ \mu\text{m}: 0$	$\geq 50\ \mu\text{m}: 0$	$\geq 50\ \mu\text{m}: 0$
Genomic Titer	ddPCR (vg/mL)	$1.99\text{E}+12$	$2.12\text{E}+12$	$1.98\text{E}+12$
Transgene Expression (Protein-based)	Cell Transduction Assay with Expression by ELISA (% Relative Potency)	121.8	142.6	125.9
Monodispersion	DLS (% 10-100 d.nm)	100	100	100

Storage at $2-8^{\circ}\text{C}$		Test Interval (Months)		
Attribute	Test Method	0	3	6
pH	Potentiometry	7.6	7.5	7.5
Subvisible Particles	Background Membrane Imaging (Particles/mL)	$\geq 10\ \mu\text{m}: 0$	$\geq 10\ \mu\text{m}: 10$	$\geq 10\ \mu\text{m}: 0$
		$\geq 25\ \mu\text{m}: 0$	$\geq 25\ \mu\text{m}: 0$	$\geq 25\ \mu\text{m}: 0$
		$\geq 50\ \mu\text{m}: 0$	$\geq 50\ \mu\text{m}: 0$	$\geq 50\ \mu\text{m}: 0$
Genomic Titer	ddPCR (vg/mL)	$1.99\text{E}+12$	$2.15\text{E}+12$	$1.95\text{E}+12$
Transgene Expression (Protein-based)	Cell Transduction Assay with Expression by ELISA (% Relative Potency)	114.6	113.7	100
Monodispersion	DLS (% 10-100 d.nm)	100	100	100

[00126] Table 11: Stability at $\leq -60^{\circ}\text{C}$ and $2-8^{\circ}\text{C}$

Storage at ≤-60°C		Test Interval (Months)		
Attribute	Test Method	0	3	12
pH	Potentiometry	7.9	7.8	7.8
Subvisible Particles	Background Membrane Imaging (Particles/mL)	≥ 10 µm: 0	≥ 10 µm: 0	≥ 10 µm: 0
		≥ 25 µm: 0	≥ 25 µm: 0	≥ 25 µm: 0
		≥ 50 µm: 0	≥ 50 µm: 0	≥ 50 µm: 0
Genomic Titer	ddPCR (vg/mL)	1.73E+12	2.00E+12	1.77E+12
Transgene Expression (Protein-based)	Cell Transduction Assay with Expression by ELISA (% Relative Potency)	122.1	141.9	124.3
Monodispersion	DLS (% 10-100 d.nm)	100	100	100

Storage at 2-8°C		Test Interval (Months)		
Attribute	Test Method	0	3	6
pH	Potentiometry	7.9	7.8	7.8
Subvisible Particles	Background Membrane Imaging (Particles/mL)	≥ 10 µm: 0	≥ 10 µm: 0	≥ 10 µm: 10
		≥ 25 µm: 0	≥ 25 µm: 0	≥ 25 µm: 0
		≥ 50 µm: 0	≥ 50 µm: 0	≥ 50 µm: 0
Genomic Titer	ddPCR (vg/mL)	1.73E+12	1.98E+12	1.71E+12
Transgene Expression (Protein-based)	Cell Transduction Assay with Expression by ELISA (% Relative Potency)	122.1	129	89.6
Monodispersion	DLS (% 10-100 d.nm)	100	100	100

[00127] Table 12: Clinical Lot Stability at $\leq -60^{\circ}\text{C}$ and $2-8^{\circ}\text{C}$

Storage at $\leq -60^{\circ}\text{C}$		Test Interval (Months)			
Attribute	Test Method	0	3	6	12
pH	Potentiometry	7.9	7.8	7.8	7.8
Subvisible Particles	Background Membrane Imaging (Particles/mL)	$\geq 10\ \mu\text{m}: 2$	$\geq 10\ \mu\text{m}: 0$	Not Tested	$\geq 10\ \mu\text{m}: 0$
		$\geq 25\ \mu\text{m}: 0$	$\geq 25\ \mu\text{m}: 0$		$\geq 25\ \mu\text{m}: 0$
		$\geq 50\ \mu\text{m}: 0$	$\geq 50\ \mu\text{m}: 0$		$\geq 50\ \mu\text{m}: 0$
Genomic Titer	ddPCR (vg/mL)	$1.90\text{E}+12$	$2.20\text{E}+12$	$1.90\text{E}+12$	$1.90\text{E}+12$
Transgene Expression (Protein-based)	Cell Transduction Assay with Expression by ELISA (% Relative Potency)	123.7	110.1	88.8	107.7
Monodispersion	DLS (% 10-100 d.nm)	100	100	100	100

Storage at $2-8^{\circ}\text{C}$		Test Interval (Months)			
Attribute	Test Method	0	3	6	12
pH	Potentiometry	7.7	Not Tested	7.6	Not Tested
Subvisible Particles	Background Membrane Imaging (Particles/mL)	$\geq 10\ \mu\text{m}: 2$	$\geq 10\ \mu\text{m}: 0$	Not Tested	$\geq 10\ \mu\text{m}: 30$
		$\geq 25\ \mu\text{m}: 0$	$\geq 25\ \mu\text{m}: 0$		$\geq 25\ \mu\text{m}: 0$
		$\geq 50\ \mu\text{m}: 0$	$\geq 50\ \mu\text{m}: 0$		$\geq 50\ \mu\text{m}: 0$
Genomic Titer	ddPCR (vg/mL)	$1.90\text{E}+12$	$2.20\text{E}+12$	$2.00\text{E}+12$	$2.00\text{E}+12$
Transgene Expression (Protein-based)	Cell Transduction Assay with Expression by ELISA (% Relative Potency)	114.9	107.9	84.6	82.3
Monodispersion	DLS (% 10-100 d.nm)	100	100	100	100

[00128] Results indicate rAAV, comprising a capsid protein of SEQ ID NO:3 and a nucleic acid comprising the nucleotide sequence of SEQ ID NO:6, formulated in 10 mM Tris, 180 mM NaCL, 0.005% Pluronic F68 is stable for more than 12-months at $\leq -60^{\circ}\text{C}$ and equal or greater than 3 months at $2-8^{\circ}\text{C}$.

Attribute	Value	Reference
Vitreous Chamber Volume (In-Vivo)	$4.649.99 \pm 0.426.54\ \text{mm}^3$ (women) $4.969.0 \pm 0.465.20\ \text{mm}^3$ (men)	Azhdam, Ariel M., et al. Vis. Sci. Tech. 2020;9(1):2.
Osmolality (living human vitreous)	$289.5 \pm 6.9\ \text{mOsm/kg}$	Kokavec J., et al. Clin Exp Ophthalmol. 2016 Sep;44(7):597-609
pH (fresh vitreous samples)	7.29 with a 95% CI of 7.20-7.39	Sobolewska et al. Int J Retin Vitro (2017) 3:22

[00129] While the materials and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the method described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention.

CLAIMS

1. A pharmaceutical composition comprising adeno-associated virus (AAV) and a buffering composition comprising:
 - (i) about 5 mM to about 20 mM of a buffering agent, preferably a Tris buffer;
 - (ii) about 100 mM to about 250 mM of a pharmaceutically acceptable salt, preferably NaCl; and
 - (iii) about 0.0001% (w/v) to about 0.01% (w/v) of a non-ionic surfactant;wherein the buffering composition has a pH of about 7.0 to about 9.0.
2. The pharmaceutical composition according to claim 1, wherein the buffering composition comprises:
 - (i) about 7 mM to about 15 mM of the buffering agent;
 - (ii) about 150 mM to about 200 mM of the pharmaceutically acceptable salt; and
 - (iii) about 0.0001% (w/v) to about 0.01% (w/v) of the non-ionic surfactant;wherein the buffering composition has a pH above 7.0.
3. The pharmaceutical composition according to claim 1 or 2, wherein the buffering composition comprises:
 - (i) about 7 mM to about 12 mM of the buffering agent;
 - (ii) about 150 to about 200 mM of the pharmaceutically acceptable salt; and
 - (iii) about 0.0005% (w/v) to about 0.01% (w/v) of the non-ionic surfactant;wherein the buffering composition has a pH above 7.0 and below about 8.5.
4. The pharmaceutical composition according to any one of claims 1-3, wherein the buffering composition comprises:
 - (i) about 7 mM to about 12 mM of the buffering agent;
 - (ii) about 150 to about 200 mM of the pharmaceutically acceptable salt; and
 - (iii) about 0.0005% (w/v) to about 0.01% (w/v) of the non-ionic surfactant;

wherein the buffering composition has a pH of about 7.5 to about 8.3.

5. The pharmaceutical composition according to claim 1, wherein the buffering composition comprises:

- (i) about 9 mM to about 20 mM of the buffering agent;
- (ii) about 140 to about 200 mM of the pharmaceutically acceptable salt; and
- (iii) about 0.001% (w/v) to about 0.01% (w/v) of the non-ionic surfactant;

wherein the buffering composition has a pH of about 7.3 to 8.6.

6. The pharmaceutical composition to any one of claims 1-5, wherein the buffering composition comprises:

- (i) about 10 mM of the buffering agent;
- (ii) about 180 mM of the pharmaceutically acceptable salt;
- (iii) about 0.005% of the nonionic surfactant; and

wherein the buffering composition has a pH of about 7.9.

7. The pharmaceutical composition according to any one of claims 1-6, wherein the buffering agent is a Tris buffer.

8. The pharmaceutical composition according to claim 7, wherein the pharmaceutical composition comprises about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM or about 20 mM Tris buffer.

9. The pharmaceutical composition according to any one of claims 1-8, wherein the pharmaceutically acceptable salt is selected from the group consisting of a sodium salt, an ammonium salt, a potassium salt and a combination thereof.

10. The pharmaceutical composition according to claim 9, wherein the pharmaceutically acceptable salt is NaCl.

11. The pharmaceutical composition according to claim 1, comprising about 100-250 mM, about 110-240 mM, about 120-230 mM, about 130-220 mM, about 140-220 mM, about

- 150-210 mM, about 150-200 mM, about 160-200 mM, about 170-190 mM, about 175-190 mM, about 175-185 mM or about 180 mM NaCl.
12. The pharmaceutical composition according to claim 1, wherein the buffering composition has a pH of about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, about 8.0, about 8.1, about 8.2, about 8.3, about 8.4, about 8.5, about 8.6 or about 8.7.
13. The pharmaceutical composition according to any one of claims 1-12, wherein the nonionic surfactant is nonionic block copolymer, preferably a polyoxyethylene-polyoxypropylene block copolymer, more preferably Pluronic F68, preferably wherein the pharmaceutical composition comprises about 0.001%, about 0.002%, about 0.003%, about 0.004%, about 0.005%, about 0.006%, about 0.007%, about 0.008%, about 0.009% or about 0.01% Pluronic F68.
14. The pharmaceutical composition according to any one of claims 1-13, wherein the pharmaceutical composition exhibits reduced subvisible particle formation and/or improved liquid storage stability compared to a pharmaceutical composition comprising phosphate-buffered saline (PBS) buffer (8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 2.7 mM KCl, 138 mM NaCl, 0.9 mM CaCl₂ and 0.5 mM MgCl₂, pH 7.0; DPBS) and Pluronic F68.
15. The pharmaceutical composition according to any one of claims 1-14, wherein the AAV comprises a capsid protein of serotype 2 (AAV2 capsid) or a variant thereof.
16. The pharmaceutical composition according to claim 15, wherein the AAV comprises a variant capsid protein comprising a heterologous peptide with a length of 7, 8, 9, 10 or 11 amino acids covalently inserted in the GH-loop of the capsid protein relative to AAV2 capsid, wherein the peptide insertion comprises the amino acid sequence ISDQTKH (SEQ ID NO:1).
17. The pharmaceutical composition according to claim 16, wherein the insertion peptide has from 1 to 3 spacer amino acids (Y₁-Y₃) at the amino and/or carboxyl terminus of amino acid sequence ISDQTKH (SEQ ID NO:1), preferably wherein the insertion peptide is LAISDQTKHA (SEQ ID NO:2).

18. The pharmaceutical composition according to claim 17, wherein the peptide is inserted following any of the amino acids in positions 584-591 in VP1 of AAV2 or a corresponding position in another AAV serotype, preferably wherein the insertion site is between amino acids 587 and 588 of VP1 of AAV2 or is between amino acids 588 and 589 of AAV2 or the corresponding positions in the capsid protein of another AAV serotype.
19. The pharmaceutical composition according to any one of claims 16-18, wherein the variant capsid protein comprises one or more amino acid substitution(s) relative to VP1 of AAV2, preferably one or more of the following amino acid substitutions: MIL, L15P, P34A, N57D, N66K, R81Q, Q101R, S109T, R144K, R144M, Q164K, T176P, L188I, S196Y, G226E, G236V, I240T, P250S, N312K, P363L, D368H, N449D, T456K, S463Y, D472N, R484C, A524T, P535S, N551S, A593E, 1698V, V708I, V719M, S721L, and L735Q, more preferably a P34A amino acid substitution.
20. The pharmaceutical composition according to claim 19, wherein the variant capsid protein is at least 90% identical, at least 95% identical, at least 98% identical or 100% identical to the entire length of the amino acid sequence set forth as SEQ ID NO:3.
21. The pharmaceutical composition according to any one of claims 1-20, wherein the AAV is a recombinant AAV (rAAV) comprising a heterologous nucleic acid comprising a nucleotide sequence encoding a gene product.
22. The pharmaceutical composition according to claim 21, wherein the gene product is an interfering RNA and/or a polypeptide and wherein the nucleotide sequence encoding the gene product is operably linked to a promoter.
23. The pharmaceutical composition according to claim 22, wherein the heterologous nucleic acid comprises a nucleotide sequence encoding a polypeptide of SEQ ID NO:5.
24. The pharmaceutical composition according to claim 23, wherein the nucleotide sequence encoding a polypeptide of SEQ ID NO:5 is at least 80%, at least 85%, at last 90%, at least 95%, or is 100% identical to SEQ ID NO:4.
25. The pharmaceutical composition according to any one of claims 22-24, wherein the nucleotide sequence encodes an interfering RNA that reduces the expression of VEGF-C.

26. The pharmaceutical composition according to any one of claims 23-25, wherein the heterologous nucleic acid comprises a nucleotide sequence at least 70% identical to the nucleotide sequence set forth as SEQ ID NO:6.
27. The pharmaceutical composition according to any one of claims 23-26, for use in the treatment of neovascular (wet) age-related macular degeneration, macular edema following retinal vein occlusion, diabetic macular edema and/or diabetic retinopathy.
28. The pharmaceutical composition according to claim 22, wherein the heterologous nucleic acid comprises a nucleotide sequence encoding a Rab escort protein-1 (REP1) protein.
29. The pharmaceutical composition according to claim 28, wherein the nucleotide sequence encoding the REP1 protein is at least 80%, at least 85%, at least 90%, at least 95%, or is 100% identical to SEQ ID NO:7.
30. The pharmaceutical composition according to claim 28 or 29, for use in the treatment of choroideremia.
31. The pharmaceutical composition according to claim 22, wherein the heterologous nucleic acid comprises a nucleotide sequence encoding a Retinitis Pigmentosa GTPase Regulator (RPGR) protein.
32. The pharmaceutical composition according to claim 31, wherein the nucleotide sequence encoding the RPGR protein is at least 80%, at least 85%, at least 90%, at least 95%, or is 100% identical to SEQ ID NO:8.
33. The pharmaceutical composition according to claim 31 or 32, for use in the treatment of X-linked retinitis pigmentosa.
34. The pharmaceutical composition according to claim 22, wherein the heterologous nucleic acid comprises a nucleotide sequence encoding a complement regulator factor H (CFH) protein or a fragment thereof.
35. The pharmaceutical composition according to claim 34, wherein the nucleotide sequence encoding the CFH protein or fragment thereof has at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% sequence identity to the amino acid sequence set forth in SEQ ID NO:9.

36. The pharmaceutical composition according to claim 35, wherein the nucleotide sequence encoding the CFH protein or fragment thereof comprises the amino acid sequence set forth in SEQ ID NO:9.
37. The rAAV according to any one of claims 34-36, wherein the rAAV comprises a heterologous nucleic acid comprising a nucleotide sequence with at least 80%, at least 85%, at least 90%, at least 95%, at least 98% sequence identity to the nucleotide sequence set forth in any one of SEQ ID NOs:10-13 or is identical to the nucleotide sequence set forth in any one of SEQ ID Nos:10-13.
38. The pharmaceutical composition according to any one of claims 34-37, for use in the treatment of dry age-related macular degeneration and/or geographic atrophy.
39. The pharmaceutical composition according to any one of claims 21-38, wherein the nucleic acid further comprises a 5' ITR, a promoter operably linked to said nucleotide sequence, a polyadenylation sequence and a 3' ITR.
40. The pharmaceutical composition according to claim 39, wherein the promoter is a ubiquitous promoter, preferably wherein the promoter is a CAG or CBA promoter.
41. The pharmaceutical composition according to claim 39, wherein the promoter is a tissue-specific promoter, preferably wherein the promoter is a rhodopsin kinase promoter.
42. The pharmaceutical composition according to any one of claim 1-41, wherein the pharmaceutical composition comprises a unit dose of 1×10^8 to 1×10^{15} vector particles (vp), preferably between 1×10^{10} to 1×10^{13} vp or between 1×10^{11} to 1×10^{12} vp.
43. The pharmaceutical composition according to any one of claims 1-42, wherein the pharmaceutical composition is a storage stable composition.
44. The pharmaceutical composition according to claim 43, wherein the pharmaceutical composition is stable as a liquid for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or at least 24 months at a temperature of less than or equal to about -60°C.
45. The pharmaceutical composition according to claim 43 or 44, wherein the pharmaceutical composition is stable as a liquid for at least two weeks, at least 3 weeks, at least one month, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 2 months, at least 9

weeks, at least 10 weeks, at least 11 weeks, at least 3 months, at least 13 weeks, at least 14 weeks, at least 15 weeks, at least 4 months, at least 17 weeks, at least 18 weeks, at least 19 weeks, at least 5 months or at least 6 months at a temperature of about 2-8°C.

46. The pharmaceutical composition according to any one of claims 43-45, wherein the pharmaceutical composition maintains a relative potency in liquid form of at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or at least 24 months.
47. The pharmaceutical composition according to any one of claims 43-46, wherein the buffering composition comprises about 10 mM Tris, about 180 mM NaCL, about 0.005% Pluronic F68 and wherein the pH of the buffering composition is about 7.4 to about 8.4 at 25 °C.
48. The pharmaceutical composition according to claim 47, wherein the pH of the buffering composition is about 7.6 to about 8.2 at 25 °C.
49. The pharmaceutical composition according to any one of claims 1-48, wherein the pharmaceutical composition does not comprise a divalent cation and does not comprise a sugar or sugar alcohol.