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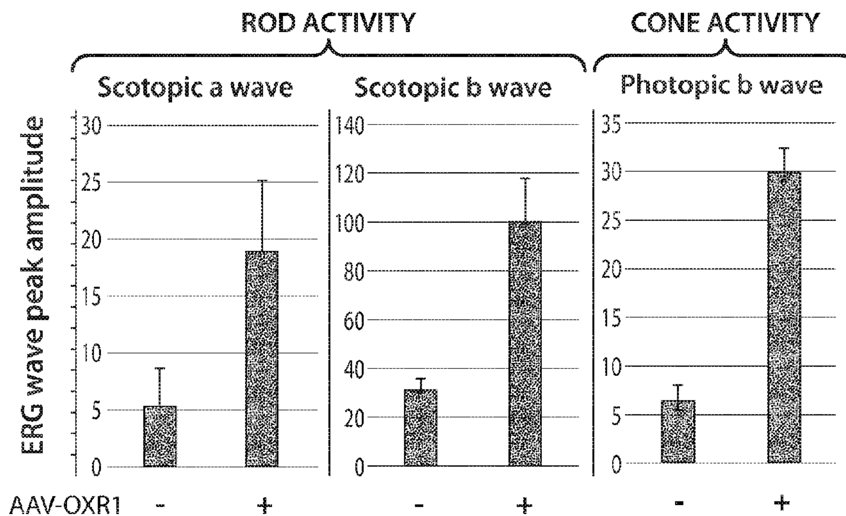

FIG. 2A

(57) Abstract: Aspects of the disclosure relate to isolated nucleic acids, rAAVs, and compositions configured to express an oxidative stress resistance protein (e.g., OXR1, NCOA7-AS, NCOA7-FL). In some embodiments, the compositions of the disclosure are useful for treatment of diseases or conditions associated with oxidative stress, for example neuronal degeneration.

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OXR1 GENE THERAPY

RELATED APPLICATION

This application claims the benefit under 35 U.S.C. § 119(e) of the filing date of U.S. Provisional Application Serial No. 62/809,021, entitled "OXR1 GENE THERAPY" and filed on February 22, 2019, the entire contents of which are incorporated herein by reference.

BACKGROUND

Reactive oxygen species (ROS) are produced as a by-product of oxygen metabolism and can cause cell damage and death. Antioxidants such as glutathione peroxidase and superoxide dismutase break down ROS before they damage proteins, DNA, RNA, and lipids. Oxidative stress is an imbalance between the amount of ROS produced and broken down. Prolonged oxidative stress results in cell damage and death and is associated with diseases such as aging, retinitis pigmentosa, age-related macular degeneration, diabetes, and neurodegenerative disease such as amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, and lupus.

SUMMARY

Aspects of the disclosure relate to compositions (*e.g.*, nucleic acids, rAAVs, *etc.*) that are configured to express one or more oxidative stress resistance proteins (*e.g.*, OXR1, NCOA7-AS and/or NCOA7-FL). In some embodiments, compositions described by the disclosure are useful for decreasing oxidative stress in cell and/or inhibiting neuronal cell (*e.g.*, ocular neuronal cell) degeneration. In some aspects, the disclosure relates to methods of treating diseases and disorders associated with neuronal cell (*e.g.*, ocular neuronal cell) degeneration, for example retinitis pigmentosa, age-related macular degeneration, retinopathy of prematurity, diabetic retinopathy, *etc.*

In some aspects, the disclosure provides an isolated nucleic acid comprising a transgene comprising a sequence as set forth in any one of SEQ ID NOs: 8-14 or 32-40 flanked by two adeno-associated virus (AAV) inverted terminal repeats (ITRs). In some aspects, the disclosure provides an isolated nucleic acid comprising a transgene encoding a protein having an amino acid sequence as set forth in any one of SEQ ID NOs: 15-22 or 31.

In some embodiments, a transgene encodes a sequence that is at least 70% (e.g., at least 70%, 80%, 90%, 95%, 99%, etc.) identical to a nucleotide sequence as set forth in any one of

SEQ ID NOs: 8-14 or 32-40. In some embodiments, a transgene encodes a sequence that is at least 70% (e.g., at least 70%, 80%, 90%, 95%, 99%, etc.) identical to an amino acid sequence as set forth in any one of SEQ ID NOs: 15-22.

In some embodiments, a transgene is operably linked to a promoter. In some embodiments, the promoter is a tissue-specific promoter or a constitutive promoter. In some embodiments, a tissue-specific promoter is specific for ocular tissue.

In some embodiments, a transgene is flanked by adeno-associated virus (AAV) inverted terminal repeats (ITR). In some embodiments, at least one of the AAV ITRs flanking a transgene lacks a functional terminal resolution site (TRS). In some embodiments, AAV ITRs are AAV2 ITRs. In some embodiments, an rAAV vector comprises the sequence set forth in any one of SEQ ID NOs: 23-30.

In some embodiments, an isolated nucleic acid is contained in a vector. In some embodiments, the vector is a plasmid or a Baculovirus vector.

In some aspects, the disclosure provides a recombinant AAV (rAAV) comprising an isolated nucleic acid as described by the disclosure and an AAV capsid protein. In some embodiments, a rAAV is a self-complementary AAV (scAAV).

In some embodiments, an AAV capsid protein has a tropism for ocular cells. In some embodiments, an AAV capsid protein is an AAV8 capsid protein.

In some aspects, the disclosure provides a composition comprising an isolated nucleic acid or the rAAV as described by the disclosure. In some embodiments, a composition comprises a pharmaceutically-acceptable excipient.

In some aspects, the disclosure provides a host cell comprising an isolated nucleic acid or the rAAV as described by the disclosure. In some embodiments, the host cell is a bacterial cell, a mammalian cell, or an insect cell. In some embodiments, a mammalian cell is a photoreceptor cell or an ocular cell (*e.g.*, retinal cell, corneal cell, optic nerve cell, *etc.*).

In some aspects, the disclosure provides a method of inhibiting neuronal cell degeneration in a subject comprising administering to the subject an isolated nucleic acid, rAAV, or composition as described by the disclosure in an amount effective to inhibit neuronal cell degeneration (*e.g.*, inhibited relative to a subject that has not been administered the rAAV).

In some embodiments, the cells are photoreceptor cells, pigmented retinal epithelial cells, neurons, or glial cells. In some embodiments, OXR1 mediated gene therapy is useful for

the treatment of oxidative stress induced damage to tissues other than neurons (*e.g.*, heart, lung, liver, or other cell types subject to oxidative stress induced damage and death).

In some embodiments, a subject has or is suspected of having a disease associated with neuronal cell degeneration. In some embodiments, the disease is associated with degeneration of ocular neuronal cells (*e.g.*, photoreceptor cells).

In some embodiments, the rAAV is administered to the subject intraocular injection, subretinal injection, intraneural injection, intrarenal injection, intravenous injection, intramuscular injection, or infusion.

In some embodiments, neuronal cell degeneration is inhibited between 2-fold and 100-fold (*e.g.*, any integer between 2 and 100, inclusive) following the administration.

In some embodiments, the disease is retinitis pigmentosa, age-related macular degeneration, retinopathy of prematurity, diabetic retinopathy, or neurodegenerative disorders (*e.g.,* amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Parkinson's disease, Huntington's disease, and lupus).

In some aspects, the disclosure provides a method for treating a disease or disorder associated with photoreceptor cell degeneration in a subject comprising administering to the subject an isolated nucleic acid, rAAV, or composition as described by the disclosure. In some embodiments, the disease is retinitis pigmentosa.

In some embodiments, methods described by the disclosure further comprise measuring photoreceptor cell activity in a subject. In some embodiments, the photoreceptor cell activity is measured by electroretinography (ERG). In some embodiments, morphological changes are determined by optical coherence tomography (OCT), fundoscopy, or by histological examination of retinal tissue. In some embodiments, behavioral assays, such as optomotor tests, are used to test visual function.

In some embodiments, after the administration, the subject has between 3.5-fold and 100-fold higher peak scotopic a wave activity relative to an untreated subject. In some embodiments, after the administration, the subject has between 3.5-fold higher and 200-fold higher peak scotopic b wave activity relative to an untreated subject. In some embodiments, after the administration, the subject has between 4.8-fold and 400-fold higher level in the peak photopic b wave activity relative to an untreated subject.

In some embodiments, the administration is intraocular injection, subretinal injection, intraneural injection, intrarenal injection, intravenous injection, intramuscular injection, or infusion.

In some embodiments, administration of the isolated nucleic acid, the rAAV, or the composition results in transduction of neuronal cells (*e.g.*, ocular neuronal cells), retinal cells (*e.g.*, bipolar cells, ganglion cells, horizontal cells, amacrine cells, *etc.*), or photoreceptor cells.

In some aspects, the disclosure provides a method for inhibiting oxidative stress in a cell comprising contacting the cell with an isolated nucleic acid, rAAV, or composition as described by the disclosure in an amount sufficient to reduce reactive oxygen species (ROS) in the cell. In some embodiments, the ROS are selected from superoxide radicals, hydroxyl radicals, peroxides, and singlet oxygen.

In some embodiments, the cell is a neuronal cell, a photoreceptor cell, a pigmented retinal epithelial cell, or a glial cell. In some embodiments, the cell is in a subject. In some embodiments, the subject has a disease associated with neuronal degeneration. In some embodiments, the subject has a disease associated with ocular cell degeneration.

In some aspects, the disclosure provides a kit comprising a container enclosing the an isolated nucleic acid, rAAV, or composition as described by the disclosure. In some embodiments, the container is a syringe.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 shows the cell survival of wild-type 661W photoreceptor cone cells and 661W photoreceptor cone cells that overexpress *OXR1* in response to treatment with hydrogen peroxide (H_2O_2).

FIGs. 2A-2C show electroretinography (ERG) data for retinal degeneration (RD1) mutant mice that express OXR1 (RD1 + OXR1) and control RD1 mutant mice (RD1 Ctrl). **FIG. 2A** shows the scotopic a wave amplitude, scotopic b wave amplitude, and photopic b wave amplitude of mice expressing OXR1 (+) and control mice (-). **FIG 2B** shows the increase in ERG amplitudes of scotopic b waves after a 12-week duration. **FIG 2C** shows the increase in ERG amplitudes of photopic b waves after a 12-week duration.

FIG. 3 shows a schematic depicting exons present in the OXR1 genomic DNA and the OXR1-A1, OXR1-A2, OXR1-B1, OXR1-B2, OXR1-D1, and OXR1-D2 gene isoforms.

FIG. 4 shows a schematic depicting exons present in the NCOA7 genomic DNA and the NCOA7-FL and NCOA7-AS gene isoforms.

DETAILED DESCRIPTION

5 Aspects of the disclosure relate to methods and compositions for expressing a transgene encoding one or more oxidative stress resistance proteins (*e.g.,* OXR1, NCOA7-AS, and/or NCOA7-FL) in a cell or subject. In some embodiments, the transgene encodes an isolated nucleic acid. In some embodiments, the isolated nucleic acid is comprised in a recombinant adeno-associated virus (rAAV).

10 In some aspects, the disclosure relates to methods for inhibiting neuronal cell degradation by expressing a transgene encoding an oxidative stress resistance protein (*e.g.*, OXR1, NCOA7-AS, and/or NCOA7-FL) in a cell or subject. Methods and compositions described by the disclosure may be utilized, in some embodiments, to treat diseases and disorders associated with neuronal cell degradation (*e.g.*, ocular neuronal cell degeneration), for 15 example retinitis pigmentosa, age-related macular degeneration, retinopathy of prematurity, or diabetic retinopathy.

Oxidative Stress Resistance Proteins

The disclosure is based, in part, on isolated nucleic acids comprising a transgene 20 encoding one or more (*e.g.*, 1, 2, 3, 4, 5, or more) oxidative stress resistance proteins. As used herein, "an oxidative stress resistance protein" refers to a protein that prevents or decreases cellular damage caused by reactive oxygen species. Reactive oxygen species (ROS) are chemically reactive species containing oxygen that are produced as a by-product of oxygen metabolism. Examples of ROS include peroxides, superoxide, hydroxyl radical, singlet oxygen, 25 alpha-oxygen, *etc*. Antioxidants (*e.g.,* glutathione, superoxide dismutase) are produced by the body to neutralize ROS. When ROS levels rise as a result of cellular stress (*e.g.,* ultraviolet light, chemical toxicity, heat exposure, ionizing radiation), the ROS species can cause damage to DNA, RNA, lipids, and proteins. As used herein, "oxidative stress" refers to an imbalance ROS and antioxidants, resulting in cell damage and death (*e.g.*, apoptosis).

30 In some embodiments, an oxidative stress resistance protein is oxidation resistance 1 (OXR1). In some embodiments, an oxidative stress resistance protein is a nuclear receptor coactivator 7 protein. In some embodiments, an oxidative stress resistance protein is a nuclear

receptor coactivator 7-alternative start (NCOA7-AS) protein. In some embodiments, an oxidative stress resistance protein is a nuclear receptor coactivator 7-full length (NCOA7-FL) protein. In some embodiments, an oxidative stress resistance protein is a TBC1 Domain Family, Member 24 (TBC1D24). In some embodiments, a nucleic acid sequence encoding an oxidative stress resistance protein (*e.g.*, OXR1, NCOA-7-AS, NCOA-7-FL, *etc.*) is codon optimized, for example codon optimized for expression in mammalian cells or bacterial cells. In some embodiments, an amino acid sequence encoding an oxidative stress resistance protein (*e.g.*, OXR1, NCOA-7-AS, NCOA-7-FL, *etc.*) comprises one or more amino acid substitutions (*e.g.*, conservative amino acid substitutions, *etc.*) relative to an amino acid sequence encoding a wild-type oxidative stress resistance protein.

In humans, OXR1 is encoded by the OXR1 gene (Gene ID: 55074, human). The OXR1 gene in humans is ubiquitously expressed, for example in cells of neuronal tissue, adrenal tissue, and reproductive tissue.

In some embodiments, an OXR1 protein is encoded is encoded by a human OXR1 gene, which comprises the nucleic acid sequence set forth in NCBI Ref. Seq ID No: NM_001198532.1, NM_001198533.1, NM_001198534.1, NM_001198535.1, NM_018002.3, or NM_181354.4. In some embodiments, an OXR1 protein comprises an amino acid sequence that is 99% identical, 95% identical, 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to the amino acid sequence encoded by the nucleic acid sequence set forth in any one of NCBI Ref. Seq ID Nos. NM_001198532.1, NM_001198533.1, NM_001198534.1, NM_001198535.1, NM_018002.3, and NM_181354.4.

In some embodiments, an OXR1 protein is encoded by a mouse OXR1 gene, which comprises the sequence set forth in NCBI Ref Seq ID No: NM_001130163.1, NM_001130164.1, NM_001130165.1, NM_001130166.1, NM_001358976.1, NM_001358977.1, NM_001358978.1, or NM_130885.2. In some embodiments, an OXR1 protein comprises an amino acid sequence that is 99% identical, 95% identical, 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to the amino acid sequence encoded by the nucleic acid sequence set forth in NCBI Ref. Seq ID No: NM_001130163.1, NM_001130164.1, NM_001130165.1, NM_001130166.1, NM_001358976.1, NM_001358977.1, NM_001358978.1, or NM_130885.2.

In some embodiments, an OXR1 gene comprises a nucleotide sequence that is 99% identical, 95% identical, 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to the nucleic acid sequence set forth in any one of SEQ ID NOs: 8-13.

In some embodiments, a human OXR1 protein comprises an amino acid sequence set forth in NCBI Ref. Seq ID No: NP_001185461.1, NP_001185462.1, NP_001185463.1, NP_001185464.1, NP_060472.2, or NP_851999.2. In some embodiments, an OXR1 protein comprises an amino acid sequence that is 99% identical, 95% identical, 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to the amino acid sequence set forth in any one of NCBI Ref. Seq ID No: NP_001185461.1, NP_001185462.1, NP_001185463.1, NP_001185464.1, NP_060472.2, and NP_851999.2.

In some embodiments, a human OXR1 protein comprises the amino acid sequence set forth in any one of SEQ ID NOs: 15-20. In some embodiments a human OXR1 protein comprises an amino acid sequence that is 99% identical, 95% identical, 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 15-20.

In some embodiments, a mouse OXR1 protein comprises the sequence set forth in NCBI Ref. Seq ID No: NP_001123635.1, NP_001123636.1, NP_001123637.1, NP_001123638.1, NP_001345905.1, NP_001345906.1, NP_001345907.1, or NP_570955.1.

Aspects of the disclosure relate to isolated nucleic acids encoding an NCOA7 protein (*e.g.*, NCOA7-alternative start or NCOA7-full length). In some embodiments, an NCOA7 protein is a full length NCOA7 (NCOA7-FL). In some embodiments, an NCOA7 protein is an NCOA7 with an alternative start (NCOA7-AS). In some embodiments, a NCOA7 (NCOA7-AS or NCOA7-FL) protein is encoded by the NCOA7 gene (Gene ID: 135112, human). The NCOA7 gene in human is ubiquitously expressed in tissues such as nervous, adrenal, and urinary bladder.

In some embodiments, a NCOA7 (NCOA7-AS or NCOA7-FL) protein is encoded is encoded by a human NCOA7 gene, which comprises the sequence set forth in NCBI Ref. Seq ID No: NM_001122842.2, NM_001199619.1, NM_001199620.1, NM_001199621.1, NM_001199622.1, or NM_181782.5. In some embodiments, a NCOA7 (NCOA7-AS or NCOA7-FL) protein comprises an amino acid sequence that is 99% identical, 95% identical, 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to the amino acid sequence encoded by the nucleic acid sequence set forth in any one of NCBI Ref. Seq ID Nos.

NM_001122842.2, NM_001199619.1, NM_001199620.1, NM_001199621.1, NM_001199622.1, or NM_181782.5.

In some embodiments, a NCOA7 (NCOA7-AS or NCOA7-FL) protein is encoded by a mouse NCOA7 gene, which comprises the sequence set forth in NCBI Ref Seq ID No:
5 NM_001111267.2, NM_001358841.1, NM_001358842.1, or NM_172495.6. In some embodiments, a NCOA7 protein comprises an amino acid sequence that is 99% identical, 95% identical, 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to the amino acid sequence encoded by the nucleic acid sequence set forth in NCBI Ref. Seq ID No: NM_001111267.2, NM_001358841.1, NM_001358842.1, or NM_172495.6.

10 In some embodiments, a NCOA7 gene comprises a nucleotide sequence that is 99% identical, 95% identical, 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to the nucleic acid sequence set forth in any one of SEQ ID NOs: 14, 39, or 40.

In some embodiments, a human NCOA7 (NCOA7-AS or NCOA7-FL) protein comprises the amino acid sequence set forth in NCBI Ref. Seq ID No: NP_001116314.1,
15 NP_001186548.1, NP_001186549.1, NP_001186550.1, NP_001186551.1, or NP_861447.3. In some embodiments, a NCOA7-AS protein comprises an amino acid sequence that is 99% identical, 95% identical, 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to the amino acid sequence encoded by the nucleic acid sequence set forth in any one of NCBI Ref. Seq ID No: NP_001116314.1, NP_001186548.1, NP_001186549.1,
20 NP_001186550.1, NP_001186551.1, or NP_861447.3.

In some embodiments, a human NCOA7 (NCOA7-AS or NCOA7-FL) protein comprises the amino acid sequence set forth in any one of SEQ ID NOs: 21, 22, or 31. In some embodiments a human NCOA7 (NCOA7-AS or NCOA7-FL) protein comprises an amino acid sequence that is 99% identical, 95% identical, 90% identical, 80% identical, 70% identical, 60%
25 identical, or 50% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 21, 22, or 31.

In some embodiments, a mouse NCOA7 (NCOA7-AS or NCOA7-FL) protein comprises the sequence set forth in NCBI Ref. Seq ID No: NP_001104737.1, NP_001345770.1, NP_001345771.1, or NP_766083.3.

30 In some embodiments, an isolated nucleic acid of the disclosure may comprise a transgene encoding one or more (*e.g.*, 1, 2, 3, 4, 5, or more) coding sequences. As used herein a "coding sequence" is the nucleotide sequence between two inverted terminal repeats (ITRs). In

some embodiments, the coding sequence encodes a protein (*e.g.,* oxidative stress resistance protein). In some embodiments, the coding sequence encodes a promoter. In some embodiments, the isolated nucleic acid transgene comprises two coding sequences. In some embodiments, the isolated nucleic acid comprises three coding sequences. In some embodiments, the isolated nucleic acid comprises four coding sequences. In some embodiments, the isolated nucleic acid comprises five coding sequences. In some embodiments, the isolated nucleic acid comprises six coding sequences. In some embodiments, the isolated nucleic acid comprises seven coding sequences. In some embodiments, the isolated nucleic acid comprises eight coding sequences. In some embodiments, the isolated nucleic acid comprises nine coding sequences. In some embodiments, the isolated nucleic acid comprises ten coding sequences.

It should be appreciated that in cases where an isolated nucleic acid transgene encodes more than one coding sequence, each coding sequence may be positioned in any suitable location within the isolated nucleic acid. For example, a nucleic acid encoding a first coding sequence (*e.g.,* OXR1, NCOA7-AS, NCOA7-FL) may be positioned in an intron of the transgene and a nucleic acid sequence encoding a second coding sequence (*e.g.* OXR1, NCOA7-AS, NCOA7-FL) may be positioned in another untranslated region (*e.g.,* between the last codon of a protein coding sequence and the first base of the poly-A tail of the transgene).

In some embodiments, the isolated nucleic acid transgene comprises coding sequences encoding least one (*e.g.,* 1, 2, 3, 4, 5 or more) oxidative stress resistance proteins (*e.g.,* OXR1, NCOA7-AS, NCOA7-FL). In some embodiments, the isolated nucleic acid transgene comprises coding sequences encoding at least one (*e.g.,* 1, 2, 3, 4, 5 or more) OXR1 protein isoforms (*e.g.,* OXR1A1, OXR1A2, OXR1B1, OXR1B2, OXR1D1, OXR1D2, or any combination of the foregoing). In some embodiments, the isolated nucleic acid transgene comprises coding sequences encoding at least one (*e.g.,* 1, 2, 3, 4, 5 or more) OXR1 protein isoforms and NCOA7 (*e.g.*, NCOA7-AS or NCOA7-FL). In some embodiments, the isolated nucleic acid comprises at least two coding sequences comprising at least two nucleotide sequences as set forth in SEQ ID NOs: 8-14 or 32-40. In some embodiments, the isolated nucleic acid comprises at least three coding sequences comprising at least three nucleotide sequences as set forth in SEQ ID NOs: 8-14 or 32-40. In some embodiments, the isolated nucleic acid comprises at least four coding sequences comprising at least four nucleotide sequences as set forth in SEQ ID NOs: 8-14 or 32-40. In some embodiments, the isolated nucleic acid comprises at least five coding sequences

comprising at least five nucleotide sequences as set forth in SEQ ID NOs: 8-14 or 32-40. In some embodiments, the isolated nucleic acid comprises at least six coding sequences comprising at least six nucleotide sequences as set forth in SEQ ID NOs: 8-14 or 32-40. In some embodiments, the isolated nucleic acid comprises at least seven coding sequences comprising at least seven nucleotide sequences as set forth in SEQ ID NOs: 8-14 or 32-40.

In some embodiments, an isolated nucleic acid encodes a combination of OXR1 isoform proteins. In some embodiments, a longer OXR1A or OXR1B isoform a OXR1D isoform or another isoform that localizes to different subcellular compartment than the OXR1A or OXR1B isoform. In some embodiments, an isolated nucleic acid encodes an OXR1A1 isoform and an OXR1D1 or OXR1D2 isoform. In some embodiments, an isolated nucleic acid encodes an OXR1A2 and an OXR1D1 or OXR1D2 isoform. In some embodiments, an isolated nucleic acid encodes an OXR1B1 isoform and an OXR1D1 or OXR1D2 isoform. In some embodiments, an isolated nucleic acid encodes an OXR1B2 isoform and an OXR1D1 or OXR1D2 isoform.

In some embodiments, the isolated nucleic acid comprises at least two coding sequences that are 99% identical, 95% identical 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to at least two nucleotide sequences as set forth in SEQ ID NOs: 8-14 or 32-40. In some embodiments, the isolated nucleic acid comprises at least three coding sequences that are 99% identical, 95% identical 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to at least three nucleotide sequences as set forth in SEQ ID NOs: 8-14 or 32-40. In some embodiments, the isolated nucleic acid comprises at least four coding sequences that are 99% identical, 95% identical 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to at least four nucleotide sequences as set forth in SEQ ID NOs: 8-14 or 32-40. In some embodiments, the isolated nucleic acid comprises at least five coding sequences that are 99% identical, 95% identical 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to at least five nucleotide sequences as set forth in SEQ ID NOs: 8-14 or 32-40. In some embodiments, the isolated nucleic acid comprises at least six coding sequences that are 99% identical, 95% identical 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to at least six nucleotide sequences as set forth in SEQ ID NOs: 8-14 or 32-40. In some embodiments, the isolated nucleic acid comprises at least seven coding sequences that are 99% identical, 95% identical 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to at least seven nucleotide sequences as set forth in SEQ ID NOs: 8-14 or 32-40.

In some embodiments, the isolated nucleic acid comprises at least two coding sequences comprising at least two amino acid sequences as set forth in SEQ ID NOs: 15-22. In some embodiments, the isolated nucleic acid comprises at least three coding sequences comprising at least three amino acid sequences as set forth in SEQ ID NOs: 15-22. In some embodiments, the isolated nucleic acid comprises at least four coding sequences comprising at least four amino acid sequences as set forth in SEQ ID NOs: 15-22. In some embodiments, the isolated nucleic acid comprises at least five coding sequences comprising at least five amino acid sequences as set forth in SEQ ID NOs: 15-22. In some embodiments, the isolated nucleic acid comprises at least six coding sequences comprising at least six amino acid sequences as set forth in SEQ ID NOs: 15-22. In some embodiments, the isolated nucleic acid comprises at least seven coding sequences comprising at least seven amino acid sequences as set forth in SEQ ID NOs: 15-22.

In some embodiments, the isolated nucleic acid comprises at least two coding sequences that are 99% identical, 95% identical 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to at least two amino acid sequences as set forth in SEQ ID NOs: 15-22. In some embodiments, the isolated nucleic acid comprises at least three coding sequences that are 99% identical, 95% identical 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to at least three amino acid sequences as set forth in SEQ ID NOs: 15-22. In some embodiments, the isolated nucleic acid comprises at least four coding sequences that are 99% identical, 95% identical 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to at least four amino acid sequences as set forth in SEQ ID NOs: 15-22. In some embodiments, the isolated nucleic acid comprises at least five coding sequences that are 99% identical, 95% identical 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to at least five amino acid sequences as set forth in SEQ ID NOs: 15-22. In some embodiments, the isolated nucleic acid comprises at least six coding sequences that are 99% identical, 95% identical 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to at least six amino acid sequences as set forth in SEQ ID NOs: 15-22. In some embodiments, the isolated nucleic acid comprises at least seven coding sequences that are 99% identical, 95% identical 90% identical, 80% identical, 70% identical, 60% identical, or 50% identical to at least seven amino acid sequences as set forth in SEQ ID NOs: 15-22.

In some embodiments, a transgene further encodes a selectable marker protein. As used herein, a selectable marker is a gene which has been introduced into a cell to facilitate artificial selection. Artificial selection, as used herein, refers to the division of cells or subjects which

possess a desired trait from cells or subjects which do not possess the desired trait. In some embodiments, a selectable marker protein facilitates positive selection, wherein the selectable marker provides an advantage to the cell or subject. In some embodiments, a selectable marker protein facilitates negative selection, wherein the selectable marker protein prohibits growth or survival of the cell or subject. Commonly utilized selectable proteins are antibiotic resistance genes, which allow the host cell or subject to survive in the presence of an antibiotic. Examples of antibiotic selectable markers include ampicillin resistance genes, geneticin resistance genes, hygromycin resistance genes, and neomycin genes.

In some embodiments, a transgene further encodes a reporter protein. In some embodiments, a reporter protein is used as an indication of whether a cell or subject comprises an isolated nucleic acid of the disclosure. Commonly utilized reporter proteins include green fluorescent protein (GFP), red fluorescent protein (RFP), yellow fluorescent protein (YFP), beta-galactosidase, and firefly Luciferase.

Isolated Nucleic Acids

A "nucleic acid" sequence refers to a DNA or RNA sequence. In some embodiments, proteins and nucleic acids of the disclosure are isolated. As used herein, the term “isolated” means artificially produced. As used herein, with respect to nucleic acids, the term “isolated” means: (i) amplified *in vitro* by, for example, polymerase chain reaction (PCR); (ii) recombinantly produced by cloning; (iii) purified, as by cleavage and gel separation; or (iv) synthesized by, for example, chemical synthesis. An isolated nucleic acid is one which is readily manipulable by recombinant DNA techniques well known in the art. Thus, a nucleotide sequence contained in a vector in which 5' and 3' restriction sites are known or for which polymerase chain reaction (PCR) primer sequences have been disclosed is considered isolated but a nucleic acid sequence existing in its native state in its natural host is not. An isolated nucleic acid may be substantially purified, but need not be. For example, a nucleic acid that is isolated within a cloning or expression vector is not pure in that it may comprise only a tiny percentage of the material in the cell in which it resides. Such a nucleic acid is isolated, however, as the term is used herein because it is readily manipulable by standard techniques known to those of ordinary skill in the art. As used herein with respect to proteins or peptides, the term “isolated” refers to a protein or peptide that has been isolated from its natural

environment or artificially produced (*e.g.*, by chemical synthesis, by recombinant DNA technology, *etc.*).

The isolated nucleic acids of the disclosure may be recombinant adeno-associated virus (AAV) vectors (rAAV vectors). In some embodiments, an isolated nucleic acid as described by the disclosure comprises a region (*e.g.*, a first region) comprising a first adeno-associated virus (AAV) inverted terminal repeat (ITR), or a variant thereof. The isolated nucleic acid (*e.g.*, the recombinant AAV vector) may be packaged into a capsid protein and administered to a subject and/or delivered to a selected target cell. “Recombinant AAV (rAAV) vectors” are typically composed of, at a minimum, a transgene and its regulatory sequences, and 5' and 3' AAV inverted terminal repeats (ITRs). The transgene may comprise a region encoding, for example, a protein and/or an expression control sequence (*e.g.*, a poly-A tail), as described elsewhere in the disclosure.

Generally, ITR sequences are about 145 bp in length. Preferably, substantially the entire sequences encoding the ITRs are used in the molecule, although some degree of minor modification of these sequences is permissible. The ability to modify these ITR sequences is within the skill of the art. (See, *e.g.*, texts such as Sambrook et al., "Molecular Cloning. A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory, New York (1989); and K. Fisher et al., J Virol., 70:520 532 (1996)). An example of such a molecule employed in the disclosure is a "cis-acting" plasmid containing the transgene, in which the selected transgene sequence and associated regulatory elements are flanked by the 5' and 3' AAV ITR sequences. The AAV ITR sequences may be obtained from any known AAV, including presently identified mammalian AAV types. In some embodiments, the isolated nucleic acid further comprises a region (*e.g.*, a second region, a third region, a fourth region, *etc.*) comprising a second AAV ITR. In some embodiments, an isolated nucleic acid encoding a transgene is flanked by AAV ITRs (*e.g.*, in the orientation 5’-ITR-transgene-ITR-3’). In some embodiments, the AAV ITRs are AAV2 ITRs.

In addition to the major elements identified above for the recombinant AAV vector, the vector also includes conventional control elements which are operably linked with elements of the transgene in a manner that permits its transcription, translation and/or expression in a cell transfected with the vector or infected with the virus produced by the disclosure. As used herein, "operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest. Expression control sequences include appropriate transcription initiation,

termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation (polyA) signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*e.g.*, Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance secretion of the encoded product. A number of expression control sequences, including promoters which are native, constitutive, inducible and/or tissue-specific, are known in the art and may be utilized.

As used herein, a nucleic acid sequence (*e.g.*, coding sequence) and regulatory sequences are said to be operably linked when they are covalently linked in such a way as to place the expression or transcription of the nucleic acid sequence under the influence or control of the regulatory sequences. If it is desired that the nucleic acid sequences be translated into a functional protein, two DNA sequences are said to be operably linked if induction of a promoter in the 5' regulatory sequences results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequences, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a promoter region would be operably linked to a nucleic acid sequence if the promoter region were capable of effecting transcription of that DNA sequence such that the resulting transcript might be translated into the desired protein or polypeptide. Similarly two or more coding regions are operably linked when they are linked in such a way that their transcription from a common promoter results in the expression of two or more proteins having been translated in frame. In some embodiments, operably linked coding sequences yield a fusion protein.

A region comprising a transgene (*e.g.*, comprising an OXR1 gene, comprising a NCOA7 gene, *etc.*) may be positioned at any suitable location of the isolated nucleic acid that will enable expression of the at least one transgene, the selectable marker protein, or reporter protein.

It should be appreciated that in cases where a transgene encodes more than one polypeptide (*e.g.,* OXR1, NCOA7-AS, NCOA7-FL), each polypeptide may be positioned in any suitable location within the transgene. For example, a nucleic acid encoding a first polypeptide may be positioned in an intron of the transgene and a nucleic acid sequence encoding a second polypeptide may be positioned in another untranslated region (*e.g.*, between the last codon of a protein coding sequence and the first base of the poly-A signal of the transgene).

A "promoter" refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. The phrases "operatively linked," "operatively positioned," "under control" or "under transcriptional control" means that the promoter is in the correct location and orientation in 5 relation to the nucleic acid to control RNA polymerase initiation and expression of the gene.

For nucleic acids encoding proteins, a polyadenylation sequence generally is inserted following the transgene sequences and before the 3' AAV ITR sequence. A rAAV construct useful in the disclosure may also contain an intron, desirably located between the promoter/enhancer sequence and the transgene. One possible intron sequence is derived from 10 SV-40, and is referred to as the SV-40 T intron sequence. Another vector element that may be used is an internal ribosome entry site (IRES). An IRES sequence is used to produce more than one polypeptide from a single gene transcript. An IRES sequence would be used to produce a protein that contain more than one polypeptide chains. Selection of these and other common vector elements are conventional and many such sequences are available [see, *e.g.*, Sambrook et 15 al., and references cited therein at, for example, pages 3.18 3.26 and 16.17 16.27 and Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989]. In some embodiments, a Foot and Mouth Disease Virus 2A sequence is included in polyprotein; this is a small peptide (approximately 18 amino acids in length) that has been shown to mediate the cleavage of polyproteins (Ryan, M D et al., EMBO, 1994; 4: 928-933; Mattion, N M et al., J 20 Virology, November 1996; p. 8124-8127; Furler, S et al., Gene Therapy, 2001; 8: 864-873; and Halpin, C et al., The Plant Journal, 1999; 4: 453-459). The cleavage activity of the 2A sequence has previously been demonstrated in artificial systems including plasmids and gene therapy vectors (AAV and retroviruses) (Ryan, M D et al., EMBO, 1994; 4: 928-933; Mattion, N M et al., J Virology, November 1996; p. 8124-8127; Furler, S et al., Gene Therapy, 2001; 8: 864-873; 25 and Halpin, C et al., The Plant Journal, 1999; 4: 453-459; de Felipe, P et al., Gene Therapy, 1999; 6: 198-208; de Felipe, P et al., Human Gene Therapy, 2000; 11: 1921-1931.; and Klump, H et al., Gene Therapy, 2001; 8: 811-817).

Examples of constitutive promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus 30 (CMV) promoter (optionally with the CMV enhancer) [see, *e.g.*, Boshart et al., Cell, 41:521-530 (1985)], the SV40 promoter, the dihydrofolate reductase promoter, the β-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1α promoter [Invitrogen]. In some

embodiments, a promoter is an RNA pol II promoter. In some embodiments, a promoter is an RNA pol III promoter, such as U6 or H1. In some embodiments, a promoter is an RNA pol II promoter. In some embodiments, a promoter is a chicken β-actin (CBA) promoter.

Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, *e.g.*, acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech and Ariad. Many other systems have been described and can be readily selected by one of skill in the art. Examples of inducible promoters regulated by exogenously supplied promoters include the zinc-inducible sheep metallothionine (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system (WO 98/10088); the ecdysone insect promoter (No et al., Proc. Natl. Acad. Sci. USA, 93:3346-3351 (1996)), the tetracycline-repressible system (Gossen et al., Proc. Natl. Acad. Sci. USA, 89:5547-5551 (1992)), the tetracycline-inducible system (Gossen et al., Science, 268:1766-1769 (1995), see also Harvey et al., Curr. Opin. Chem. Biol., 2:512-518 (1998)), the RU486-inducible system (Wang et al., Nat. Biotech., 15:239-243 (1997) and Wang et al., Gene Ther., 4:432-441 (1997)) and the rapamycin-inducible system (Magari et al., J. Clin. Invest., 100:2865-2872 (1997)). Still other types of inducible promoters which may be useful in this context are those which are regulated by a specific physiological state, *e.g.,* temperature, acute phase, a particular differentiation state of the cell, or in replicating cells only.

In another embodiment, the native promoter for the transgene (*e.g.,* OXR1, NCOA7) will be used. The native promoter may be preferred when it is desired that expression of the transgene should mimic the native expression. The native promoter may be used when expression of the transgene must be regulated temporally or developmentally, or in a tissue-specific manner, or in response to specific transcriptional stimuli. In a further embodiment, other native expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be used to mimic the native expression.

In some embodiments, the regulatory sequences impart tissue-specific gene expression capabilities. In some cases, the tissue-specific regulatory sequences bind tissue-specific transcription factors that induce transcription in a tissue specific manner. Such tissue-specific regulatory sequences (*e.g.*, promoters, enhancers, *etc.*.) are well known in the art. Exemplary

tissue-specific regulatory sequences include, but are not limited to the following tissue specific promoters: retinoschisin proximal promoter, interphotoreceptor retinoid-binding protein enhancer (RS/IRBPa), rhodopsin kinase (RK), liver-specific thyroxin binding globulin (TBG) promoter, an insulin promoter, a glucagon promoter, a somatostatin promoter, a pancreatic
5 polypeptide (PPY) promoter, a synapsin-1 (Syn) promoter, a creatine kinase (MCK) promoter, a mammalian desmin (DES) promoter, a α-myosin heavy chain (α-MHC) promoter, or a cardiac Troponin T (cTnT) promoter. Other exemplary promoters include Beta-actin promoter, hepatitis B virus core promoter, Sandig et al., Gene Ther., 3:1002-9 (1996); alpha-fetoprotein (AFP) promoter, Arbuthnot et al., Hum. Gene Ther., 7:1503-14 (1996)), bone osteocalcin promoter
10 (Stein et al., Mol. Biol. Rep., 24:185-96 (1997)); bone sialoprotein promoter (Chen et al., J. Bone Miner. Res., 11:654-64 (1996)), CD2 promoter (Hansal et al., J. Immunol., 161:1063-8 (1998); immunoglobulin heavy chain promoter; T cell receptor α-chain promoter, neuronal such as neuron-specific enolase (NSE) promoter (Andersen et al., Cell. Mol. Neurobiol., 13:503-15 (1993)), neurofilament light-chain gene promoter (Piccioli et al., Proc. Natl. Acad. Sci. USA,
15 88:5611-5 (1991)), and the neuron-specific vgf gene promoter (Piccioli et al., Neuron, 15:373-84 (1995)), among others which will be apparent to the skilled artisan.

In some embodiments, a transgene which encodes at least one oxidative stress resistance protein (*e.g.,* OXR1, NCOA7-AS, NCOA7-FL) is operably linked to a promoter. In some embodiments, a transgene which encodes a selectable marker or reporter protein is operably
20 linked to a promoter. In some embodiments, the transgene encoding the at least one oxidative stress resistance protein (*e.g.,* OXR1, NCOA7-AS, NCOA7-FL) and the transgene which encodes a selectable marker or reporter protein are operably linked to the same promoter. In some embodiments, the transgene encoding the at least one oxidative stress resistance protein and the transgene which encodes a selectable marker or reporter protein are operably linked to
25 different promoters. In some embodiments, the promoter is a constitutive promoter. In some embodiments, the promoter is an inducible promoter. In some embodiments, the promoter is a tissue-specific promoter. In some embodiments, the tissue-specific promoter is an ocular tissue promoter retinoschisin proximal promoter, interphotoreceptor retinoid-binding protein enhancer (RS/IRBPa), rhodopsin kinase (RK), RPE65, and human cone opsin promoters.

30 In some embodiments, the tissue-specific promoter is a neuron-specific promoter, or a central nervous system (CNS)-specific promoter. In some embodiments, the tissue-specific promoter is a synapsin promoter, a SOD1 promoter, a Chat promoter, a GFAP promoter, a

calcium/calmodulin-dependent protein kinase II promoter, a tubulin alpha I promoter, a neuron-specific enolase promoter, or a platelet-derived growth factor beta chain promoter.

Aspects of the disclosure relate to an isolated nucleic acid comprising more than one promoter (*e.g.*, 2, 3, 4, 5, or more promoters). For example, in the context of a construct having a transgene comprising a first region (*e.g.*, OXR1, NCOA7) and a second region (*e.g.*, a selectable marker protein, reporter protein, therapeutic protein, *etc.*) it may be desirable to drive expression of the first protein coding region using a first promoter sequence (*e.g.*, a first promoter sequence operably linked to the first region), and to drive expression of the second region with a second promoter sequence (*e.g.*, a second promoter sequence operably linked to the second region). Generally, the first promoter sequence and the second promoter sequence can be the same promoter sequence or different promoter sequences. In some embodiments, the second promoter sequence (*e.g.*, the promoter sequence driving expression of the second region) is a RNA polymerase II (pol II) promoter sequence. Non-limiting examples of pol II promoter sequences include T7, T3, SP6, RSV, and cytomegalovirus promoter sequences. In some embodiments, a pol III promoter sequence drives expression of the first region. In some embodiments, a pol II promoter sequence drives expression of the second region.

Recombinant adeno-associated viruses (rAAVs)

In some aspects, the disclosure provides isolated adeno-associated viruses (AAVs). As used herein with respect to AAVs, the term "isolated" refers to an AAV that has been artificially produced or obtained. Isolated AAVs may be produced using recombinant methods. Such AAVs are referred to herein as "recombinant AAVs". Recombinant AAVs (rAAVs) preferably have tissue-specific targeting capabilities, such that a transgene of the rAAV will be delivered specifically to one or more predetermined tissue(s) (*e.g.,* ocular tissues, neurons). The AAV capsid is an important element in determining these tissue-specific targeting capabilities (*e.g.*, tissue tropism). Thus, an rAAV having a capsid appropriate for the tissue being targeted can be selected.

In some embodiments, rAAVs of the disclosure comprise a nucleotide sequence as set forth in any one of SEQ ID NOs: 1-7 or encode a protein having an amino acid sequence as set forth in any one of claims 8-14 or 32-40. In some embodiments, rAAVs of the disclosure comprise a nucleotide sequence that is 99% identical, 95% identical, 90% identical, 85%

identical, 80% identical, 75% identical, 70% identical, 65% identical, 60% identical, 55% identical, or 50% identical to a nucleotide sequence as set forth in SEQ ID NOs: 1-7.

Methods for obtaining recombinant AAVs having a desired capsid protein are well known in the art. (See, for example, US 2003/0138772), the contents of which are incorporated herein by reference in their entirety). Typically the methods involve culturing a host cell which contains a nucleic acid sequence encoding an AAV capsid protein; a functional rep gene; a recombinant AAV vector composed of AAV inverted terminal repeats (ITRs) and a transgene; and sufficient helper functions to permit packaging of the recombinant AAV vector into the AAV capsid proteins. In some embodiments, capsid proteins are structural proteins encoded by the cap gene of an AAV. AAVs comprise three capsid proteins, virion proteins 1 to 3 (named VP1, VP2 and VP3), all of which are transcribed from a single cap gene via alternative splicing. In some embodiments, the molecular weights of VP1, VP2 and VP3 are respectively about 87 kDa, about 72 kDa and about 62 kDa. In some embodiments, upon translation, capsid proteins form a spherical 60-mer protein shell around the viral genome. In some embodiments, the functions of the capsid proteins are to protect the viral genome, deliver the genome and interact with the host. In some aspects, capsid proteins deliver the viral genome to a host in a tissue specific manner.

In some embodiments, an AAV capsid protein has a tropism for ocular tissues. In some embodiments, an AAV capsid protein targets ocular cell types (*e.g.,* photoreceptor cells, retinal cells, etc.). In some embodiments, an AAV capsid protein is of an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAV9, AAV10, AAVrh10, and AAV.PHP.B. In some embodiments, an AAV capsid protein is of a serotype derived from a non-human primate, for example AAVrh8 serotype. In some embodiments, an AAV capsid protein is of a serotype derived for broad and efficient CNS transduction, for example AAV9 or AAV.PHP.B . In some embodiments, the capsid protein is of AAV serotype 8 (*e.g.*, AAV8 capsid protein), AAV serotype 2 (*e.g.*, AAV2 capsid protein), AAV serotype 5 (*e.g.*, AAV5 capsid protein), or AAV serotype 9 (*e.g.*, AAV9 capsid protein).

In some embodiments, an rAAV vector or rAAV particle comprises a mutant ITR that lacks a functional terminal resolution site (TRS). The term "lacking a terminal resolution site" can refer to an AAV ITR that comprises a mutation (e.g., a sense mutation such as a non-synonymous mutation, or missense mutation) that abrogates the function of the terminal resolution site (TRS) of the ITR, or to a truncated AAV ITR that lacks a nucleic acid sequence

encoding a functional TRS (*e.g.*, a ΔTRS ITR). Without wishing to be bound by any particular theory, a rAAV vector comprising an ITR lacking a functional TRS produces a self-complementary rAAV vector, for example as described by McCarthy (2008) Molecular Therapy 16(10):1648-1656.

5 The components to be cultured in the host cell to package a rAAV vector in an AAV capsid may be provided to the host cell in trans. Alternatively, any one or more of the required components (*e.g.*, recombinant AAV vector, *rep* sequences, *cap* sequences, and/or helper functions) may be provided by a stable host cell which has been engineered to contain one or more of the required components using methods known to those of skill in the art. Most 10 suitably, such a stable host cell will contain the required component(s) under the control of an inducible promoter. However, the required component(s) may be under the control of a constitutive promoter. Examples of suitable inducible and constitutive promoters are provided herein, in the discussion of regulatory elements suitable for use with the transgene. In still another alternative, a selected stable host cell may contain selected component(s) under the 15 control of a constitutive promoter and other selected component(s) under the control of one or more inducible promoters. For example, a stable host cell may be generated which is derived from 293 cells (which contain E1 helper functions under the control of a constitutive promoter), but which contain the rep and/or cap proteins under the control of inducible promoters. Still other stable host cells may be generated by one of skill in the art.

20 In some embodiments, the disclosure relates to a host cell containing a nucleic acid that comprises a coding sequence encoding a transgene (*e.g.,* OXR1, NCOA7). A “host cell” refers to any cell that harbors, or is capable of harboring, a substance of interest. Often a host cell is a mammalian cell. In some embodiments, a host cell is a neuron. In some embodiments, a host cell is a photoreceptor cell. A host cell may be used as a recipient of an AAV helper construct, 25 an AAV minigene plasmid, an accessory function vector, or other transfer DNA associated with the production of recombinant AAVs. The term includes the progeny of the original cell which has been transfected. Thus, a “host cell” as used herein may refer to a cell which has been transfected with an exogenous DNA sequence. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total 30 DNA complement as the original parent, due to natural, accidental, or deliberate mutation. In some embodiments, the host cell is a mammalian cell, a yeast cell, a bacterial cell, an insect cell,

a plant cell, or a fungal cell. In some embodiments, the host cell is a neuron, a photoreceptor cell, a pigmented retinal epithelial cell, or a glial cell.

The recombinant AAV vector, rep sequences, cap sequences, and helper functions required for producing the rAAV of the disclosure may be delivered to the packaging host cell using any appropriate genetic element (vector). The selected genetic element may be delivered by any suitable method, including those described herein. The methods used to construct any embodiment of this disclosure are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. See, *e.g.*, Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. Similarly, methods of generating rAAV virions are well known and the selection of a suitable method is not a limitation on the disclosure. See, *e.g.*, K. Fisher et al., J. Virol., 70:520-532 (1993) and U.S. Pat. No. 5,478,745.

In some embodiments, recombinant AAVs may be produced using the triple transfection method (described in detail in U.S. Pat. No. 6,001,650). Typically, the recombinant AAVs are produced by transfecting a host cell with an AAV vector (comprising a transgene flanked by ITR elements) to be packaged into AAV particles, an AAV helper function vector, and an accessory function vector. An AAV helper function vector encodes the "AAV helper function" sequences (*e.g.*, *rep* and *cap*), which function in trans for productive AAV replication and encapsidation. Preferably, the AAV helper function vector supports efficient AAV vector production without generating any detectable wild-type AAV virions (*e.g.*, AAV virions containing functional rep and cap genes). Non-limiting examples of vectors suitable for use with the disclosure include pHLP19, described in U.S. Pat. No. 6,001,650 and pRep6cap6 vector, described in U.S. Pat. No. 6,156,303, the entirety of both incorporated by reference herein. The accessory function vector encodes nucleotide sequences for non-AAV derived viral and/or cellular functions upon which AAV is dependent for replication (*e.g.*, "accessory functions"). The accessory functions include those functions required for AAV replication, including, without limitation, those moieties involved in activation of AAV gene transcription, stage specific AAV mRNA splicing, AAV DNA replication, synthesis of cap expression products, and AAV capsid assembly. Viral-based accessory functions can be derived from any of the known helper viruses such as adenovirus, herpes virus (other than herpes simplex virus type-1), and vaccinia virus.

In some aspects, the disclosure provides transfected host cells. The term "transfection" is used to refer to the uptake of foreign DNA by a cell, and a cell has been "transfected" when exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are generally known in the art. See, *e.g.,* Graham et al. (1973) Virology, 52:456, Sambrook et al. (1989) Molecular Cloning, a laboratory manual, Cold Spring Harbor Laboratories, New York, Davis et al. (1986) Basic Methods in Molecular Biology, Elsevier, and Chu et al. (1981) Gene 13:197. Such techniques can be used to introduce one or more exogenous nucleic acids, such as a nucleotide integration vector and other nucleic acid molecules, into suitable host cells.

As used herein, the terms “recombinant cell” refers to a cell into which an exogenous DNA segment, such as DNA segment that leads to the transcription of a biologically-active polypeptide or production of a biologically active nucleic acid such as an RNA, has been introduced.

As used herein, the term "vector" includes any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, artificial chromosome, virus, virion, *etc.*, which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. In some embodiments, a vector is a viral vector, such as an rAAV vector, a lentiviral vector, an adenoviral vector, a retroviral vector, *etc.* Thus, the term includes cloning and expression vehicles, as well as viral vectors. In some embodiments, useful vectors are contemplated to be those vectors in which the nucleic acid segment to be transcribed is positioned under the transcriptional control of a promoter.

AAV-mediated Delivery of a Transgene to Ocular Tissue

The isolated nucleic acids, rAAVs, and compositions of the disclosure may be delivered to a subject in compositions according to any appropriate methods known in the art. For example, an rAAV, preferably suspended in a physiologically compatible carrier (*e.g.*, in a composition), may be administered to a subject, i.e. host animal, such as a human, mouse, rat, cat, dog, sheep, rabbit, horse, cow, goat, pig, guinea pig, hamster, chicken, turkey, or a non-human primate (*e.g.*, Macaque). In some embodiments a host animal does not include a human.

Delivery of the rAAVs to a mammalian subject may be by, for example, intraocular injection, subretinal injection, or by injection into the eye of the mammalian subject to ocular tissues. As used herein, “ocular tissues” refers to any tissue derived from or contained in the

eye. Non-limiting examples of ocular tissues include neurons, retina (*e.g.,* photoreceptor cells), sclera, choroid, retina, vitreous body, macula, fovea, optic disc, lens, pupil, iris, aqueous fluid, cornea, conjunctiva ciliary body, and optic nerve. The retina is located in the posterior of the eye and comprises photoreceptor cells. These photoreceptor cells (*e.g.,* rods, cones) confer visual acuity by discerning color, as well as contrast in the visual field.

Moreover, in certain instances, it may be desirable to deliver the virions to the CNS of a subject. "CNS", as used herein, refers to all cells and tissue of the brain and spinal cord of a vertebrate. Thus, the term includes, but is not limited to, neuronal cells (*e.g.,* photoreceptor cells), glial cells, astrocytes, cerebrospinal fluid (CSF), interstitial spaces, bone, cartilage and the like. Recombinant AAVs may be delivered directly to the CNS or brain by injection into, *e.g.*, the ventricular region, as well as to the striatum (*e.g.*, the caudate nucleus or putamen of the striatum), thalamus, spinal cord and neuromuscular junction, or cerebellar lobule, with a needle, catheter or related device, using neurosurgical techniques known in the art, such as by stereotactic injection (see, *e.g.,* Stein et al., J Virol 73:3424-3429, 1999; Davidson et al., PNAS 97:3428-3432, 2000; Davidson et al., Nat. Genet. 3:219-223, 1993; and Alisky and Davidson, Hum. Gene Ther. 11:2315-2329, 2000).

Alternatively, delivery of the rAAVs to a mammalian subject may be by intramuscular injection or by administration into the bloodstream of the mammalian subject. Administration into the bloodstream may be by injection into a vein, an artery, or any other vascular conduit. In some embodiments, the rAAVs are administered into the bloodstream by way of isolated limb perfusion, a technique well known in the surgical arts, the method essentially enabling the artisan to isolate a limb from the systemic circulation prior to administration of the rAAV virions. A variant of the isolated limb perfusion technique, described in U.S. Pat. No. 6,177,403, can also be employed by the skilled artisan to administer the virions into the vasculature of an isolated limb to potentially enhance transduction into muscle cells or tissue. In some embodiments, an rAAV as described in the disclosure is administered by intraocular injection. In some embodiments, an rAAV as described in the disclosure is administered by subretinal injection. In some embodiments, an rAAV as described in the disclosure is administered by intravenous injection. In some embodiments, rAAVs are administered by intracerebral injection. In some embodiments, rAAVs are administered by intrathecal injection. In some embodiments, rAAVs are administered by intrastriatal injection. In some embodiments, rAAVs are delivered by intracranial injection. In some embodiments, rAAVs are delivered by

cisterna magna injection. In some embodiments, the rAAV are delivered by cerebral lateral ventricle injection.

Aspects of the instant disclosure relate to compositions comprising a recombinant AAV comprising a capsid protein and a nucleic acid encoding a transgene, wherein the transgene comprises a nucleic acid sequence encoding one or more oxidative stress resistance proteins (*e.g.,* OXR1, NCOA-7). In some embodiments, the nucleic acid further comprises AAV ITRs. In some embodiments, a composition further comprises a pharmaceutically acceptable carrier.

The compositions of the disclosure may comprise an rAAV alone, or in combination with one or more other viruses (*e.g.*, a second rAAV encoding having one or more different transgenes). In some embodiments, a composition comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more different rAAVs each having one or more different transgenes.

Suitable carriers may be readily selected by one of skill in the art in view of the indication for which the rAAV is directed. For example, one suitable carrier includes saline, which may be formulated with a variety of buffering solutions (*e.g.,* phosphate buffered saline). Other exemplary carriers include sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextran, agar, pectin, peanut oil, sesame oil, and water. The selection of the carrier is not a limitation of the disclosure.

Optionally, the compositions of the disclosure may contain, in addition to the rAAV and carrier(s), other conventional pharmaceutical ingredients, such as preservatives, or chemical stabilizers. Suitable exemplary preservatives include chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, parachlorophenol, and poloxamers (non-ionic surfactants) such as Pluronic® F-68. Suitable chemical stabilizers include gelatin and albumin.

The rAAVs are administered in sufficient amounts to transfect the cells of a desired tissue and to provide sufficient levels of gene transfer and expression without undue adverse effects. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to the selected organ (*e.g.,* intraportal delivery to the liver), intraocular injection, subretinal injection, oral, inhalation (including intranasal and intratracheal delivery), intravenous, intramuscular, subcutaneous, intradermal, intratumoral, and other parental routes of administration. Routes of administration may be combined, if desired.

The dose of rAAV virions required to achieve a particular "therapeutic effect," *e.g.*, the units of dose in genome copies/per kilogram of body weight (GC/kg), will vary based on several

factors including, but not limited to: the route of rAAV virion administration, the level of gene or RNA expression required to achieve a therapeutic effect, the specific disease or disorder being treated, and the stability of the gene or RNA product. One of skill in the art can readily determine a rAAV virion dose range to treat a patient having a particular disease or disorder based on the aforementioned factors, as well as other factors that are well known in the art.

An effective amount of an rAAV is an amount sufficient to target infect an animal, target a desired tissue. In some embodiments, an effective amount of an rAAV is administered to the subject during a pre-symptomatic stage of degenerative disease. In some embodiments, a subject is administered an rAAV or composition after exhibiting one or more signs or symptoms of degenerative disease.

An effective amount of an rAAV may also depend on the mode of administration. For example, targeting an ocular (*e.g.*, corneal) tissue by intrastromal administration or subcutaneous injection may require different (*e.g.*, higher or lower) doses, in some cases, than targeting an ocular (*e.g.*, corneal) tissue by another method (*e.g.*, systemic administration, topical administration). In some embodiments, intrastromal injection (IS) of rAAV having certain serotypes (*e.g.*, AAV2, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAVrh.8, AAVrh.10, AAVrh.39, and AAVrh.43) mediates efficient transduction of ocular (*e.g.*, corneal, retinal, *etc.*) cells. Thus, in some embodiments, the injection is intrastromal injection (IS). In some embodiments, the injection is topical administration (*e.g.*, topical administration to an eye). In some cases, multiple doses of a rAAV are administered.

Administration of compositions described by the disclosure may result in transduction of one of the foregoing eye regions, or more than one eye region (*e.g.*, 2, 3, 4, 5, or 6 eye regions). In some embodiments, administration of an rAAV as described herein results in transduction of an ocular cell type selected from the group consisting of photoreceptor cells, glial cells, basal cells, and corneal squamous cells. In some embodiments, the administration results in transduction of photoreceptor cells.

In some embodiments, rAAV compositions are formulated to reduce aggregation of AAV particles in the composition, particularly where high rAAV concentrations are present (*e.g.*, ~10^{13} GC/mL or more). Methods for reducing aggregation of rAAVs are well known in the art and, include, for example, addition of surfactants, pH adjustment, salt concentration adjustment, *etc.* (See, *e.g.*, Wright FR, et al., Molecular Therapy (2005) 12, 171–178, the contents of which are incorporated herein by reference.)

Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens.

5 Typically, these formulations may contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 70% or 80% or more of the weight or volume of the total formulation. Naturally, the amount of active compound in each therapeutically-useful composition may be prepared is such a way that a suitable dosage will be obtained in any 10 given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

In certain circumstances it will be desirable to deliver the rAAV-based therapeutic 15 constructs in suitably formulated pharmaceutical compositions disclosed herein either intraocularlly, subretinally, subcutaneously, intraopancreatically, intranasally, parenterally, intravenously, intramuscularly, intrathecally, orally, intraperitoneally, or by inhalation. In some embodiments, the administration modalities as described in U.S. Pat. Nos. 5,543,158; 5,641,515 and 5,399,363 (each specifically incorporated herein by reference in its entirety) may be used to 20 deliver rAAVs. In some embodiments, a preferred mode of administration is by portal vein injection.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and 25 mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. In many cases the form is sterile and fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion 30 medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the

maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For administration of an injectable aqueous solution, for example, the solution may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art. For example, one dosage may be dissolved in 1 mL of isotonic NaCl solution and either added to 1000 mL of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the host. The person responsible for administration will, in any event, determine the appropriate dose for the individual host.

Sterile injectable solutions are prepared by incorporating the active rAAV in the required amount in the appropriate solvent with various of the other ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The rAAV compositions disclosed herein may also be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such

organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug-release capsules, and the like.

5 As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers 10 to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a host.

Delivery vehicles such as liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, may be used for the introduction of the compositions of the disclosure into suitable host cells. In particular, the rAAV vector delivered transgenes may be 15 formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

Such formulations may be preferred for the introduction of pharmaceutically acceptable formulations of the nucleic acids or the rAAV constructs disclosed herein. The formation and use of liposomes is generally known to those of skill in the art. Recently, liposomes were 20 developed with improved serum stability and circulation half-times (U.S. Pat. No. 5,741,516). Further, various methods of liposome and liposome like preparations as potential drug carriers have been described (U.S. Pat. Nos. 5,567,434; 5,552,157; 5,565,213; 5,738,868 and 5,795,587).

Liposomes have been used successfully with a number of cell types that are normally resistant to transfection by other procedures. In addition, liposomes are free of the DNA length 25 constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, drugs, radiotherapeutic agents, viruses, transcription factors and allosteric effectors into a variety of cultured cell lines and animals. In addition, several successful clinical trials examining the effectiveness of liposome-mediated drug delivery have been completed.

30 Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs). MLVs generally have diameters of from 25 nm to 4 μm. Sonication of MLVs results in

the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

Alternatively, nanocapsule formulations of the rAAV may be used. Nanocapsules can generally entrap substances in a stable and reproducible way. To avoid side effects due to
5 intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded in vivo. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use.

In addition to the methods of delivery described above, the following techniques are also contemplated as alternative methods of delivering the rAAV compositions to a host.
10 Sonophoresis (*i.e.*, ultrasound) has been used and described in U.S. Pat. No. 5,656,016 as a device for enhancing the rate and efficacy of drug permeation into and through the circulatory system. Other drug delivery alternatives contemplated are intraosseous injection (U.S. Pat. No. 5,779,708), microchip devices (U.S. Pat. No. 5,797,898), ophthalmic formulations (Bourlais et al., 1998), transdermal matrices (U.S. Pat. Nos. 5,770,219 and 5,783,208) and feedback-
15 controlled delivery (U.S. Pat. No. 5,697,899).

Methods of treating disorders originating from oxidative stress

Prolonged oxidative stress, resulting from imbalance between production of reactive oxygen species (ROS) and antioxidant activity, results in cell damage and potentially cell death
20 due to accumulated DNA, RNA, protein, and lipid damage by ROS. Cell death due to oxidative stress is associated with numerous disorders, including ocular disorders (*e.g.*, retinitis pigmentosa, age-related macular degeneration, retinopathy of prematurity, diabetic retinopathy) and neurodegenerative disorders (*e.g.,* amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Parkinson's disease, Huntington's disease, and lupus). OXR1 and NCOA7 (*e.g.*, NCOA7-AS,
25 NCOA7-FL) protein expression is upregulated in response to oxidative stress in neurons (*e.g.,* photoreceptor cells, cranial nerves, motor neurons).

Ocular (*e.g.,* retinal) tissue can be healthy ocular (*e.g.,* retinal) tissue (*e.g.*, ocular tissue not having a disease, or at risk of developing an ocular disease, such as a retinal disease) or diseased ocular tissue (*e.g.*, ocular tissue having retinitis pigmentosa, age-related macular
30 degeneration, retinopathy of prematurity, diabetic retinopathy). As used herein, "at risk of developing an ocular disease" refers to a subject having an increased probability of developing an ocular disease (*e.g.*, retinal disease) than the general population due to the presence of a risk

factor. Examples categories of risk factors for developing ocular disease include, but are not limited to: oxidative stress, diabetes, ocular trauma, prior ocular surgery, age, race, and family history (*e.g.*, positive family history of ocular disease, high cholesterol, or high blood pressure).

In some aspects, the disclosure provides a method of inhibiting neuronal cell degeneration in a subject comprising administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject having or suspected of having a neurodegenerative disease. As used herein, a "neurodegenerative disease" is a disorder primarily affecting neurons in the central nervous system. Non-limiting examples of neurodegenerative diseases include amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), prion infections, lupus, and multiple sclerosis (MS).

In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject inhibits neuronal cell degeneration by between 2-fold and 100-fold (*e.g.*, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 75-fold, 100-fold, *etc.*) compared to a control subject. As used herein a "control" subject refers to a subject that is not administered the isolated nucleic acids, the rAAVs, or the compositions described herein. In some embodiments, a control subject is the same subject that is administered the isolated nucleic acids, the rAAVs, or the compositions described herein (*e.g.*, prior to the administration). In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described to a subject inhibits neuronal degeneration by 2-fold compared to a control. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described to a subject inhibits neuronal degeneration by 100-fold compared to a control. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described to a subject inhibits neuronal cell degeneration by 5-fold compared to a control. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described to a subject inhibits neuronal cell degeneration by 10-fold compared to a control. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described to a subject inhibits neuronal cell degeneration by 5-fold to 100-fold compared to control. (5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 45-fold, 50-fold, 55-fold, 60-fold, 65-fold, 70-fold, 75-fold, 80-fold, 85-fold, 90-fold, 95-fold, or 100-fold compared to a control).

In some aspects, the disclosure provides a method for treating a disease or disorder associated with photoreceptor cell degeneration in a subject comprising administering the

isolated nucleic acids, the rAAVs, or the compositions described herein to a subject having or suspected of being at risk of developing retinitis pigmentosa and measuring photoreceptor activity relative to a control. Retinitis pigmentosa refers to a group of genetic diseases characterized by damage to cells of the retina (*e.g.,* photoreceptors), resulting in vision loss. Autosomal recessive forms of juvenile retinitis pigmentosa can be caused by mutation in the SPATA7 (R395X), LRAT (S175R, 396AA), TULP1 (R420P, F491L, I459K, F382S, R482W), RP1 (T373I, 1461TGAA), RHO (E249T, P53R, G106R, D190Y, R207M, N15S, M207R), ABCA4 (1847A), RPE65 (P363T, L341S), EYS (17-bp DEL, NT2710), CERKL (R257T, K200T) and/or SEMA4A (D345H, F350C, R713E) genes.

In some embodiments, photoreceptor activity of a cell or subject is measured by electroretinography. Electroretinography (ERG) measures the electrical responses of various cell types in the retina (*e.g.,* photoreceptors, ganglion). In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject increases the peak scotopic a wave activity by between 3.5-fold and 100-fold (*e.g.*, about 3.5-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, *etc.*) compared to a control subject. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject increases the peak scotopic a wave activity by 3.5-fold compared to a control subject. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject increases the peak scotopic a wave activity by 100-fold compared to a control subject. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject increases the peak scotopic a wave activity by more than 100-fold (*e.g.*, 200-fold, 500-fold, 1000-fold, *etc.*) compared to a control subject. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described to a subject increases the peak scotopic a wave activity by 5-fold to 200-fold (5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 45-fold, 50-fold, 55-fold, 60-fold, 65-fold, 70-fold, 75-fold, 80-fold, 85-fold, 90-fold, 95-fold, or 100-fold compared to a control).

In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject increases the peak scotopic b wave activity by between 3.5-fold and 100-fold (*e.g.*, about 3.5-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, *etc.*) compared to a control subject. As used herein, “scotopic b wave” refers to the electrical response in the rod cells of the retina as a result of bipolar cell-depolarization. This depolarization increases the level of extracellular potassium, generating a transretinal current. In

some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject increases the peak scotopic b wave activity by 3.5-fold compared to a control subject. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject increases the peak scotopic b wave activity by 100-fold compared to a control subject. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject increases the peak scotopic b wave activity by more than 100-fold (*e.g.*, 200-fold, 500-fold, 1000-fold, *etc.*) compared to a control subject. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described to a subject increases the peak scotopic b wave activity by 3.5-fold, 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 45-fold, 50-fold, 55-fold, 60-fold, 65-fold, 70-fold, 75-fold, 80-fold, 85-fold, 90-fold, 95-fold, or 100-fold compared to a control.

In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described to a subject increases the peak photopic b wave activity by 5-fold to 400-fold (*e.g.*, about 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 300-fold, 400-fold, *etc.*). In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject increases the peak photopic b wave activity by more than 400-fold (*e.g.*, 500-fold, 800-fold, 1000-fold, *etc.*) compared to a control subject. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described to a subject increases the peak photopic b wave activity by 5-fold to 400-fold (5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 35-fold, 40-fold, 45-fold, 50-fold, 55-fold, 60-fold, 65-fold, 70-fold, 75-fold, 80-fold, 85-fold, 90-fold, 95-fold, 100-fold, 150-fold, 200-fold, 250-fold, 300-fold, 350-fold, or 400-fold compared to a control).

In some aspects, the disclosure provides a method for inhibiting oxidative stress in a cell comprising contacting the cell with the isolated nucleic acids, the rAAVs, or the compositions described herein. In some aspects, the disclosure is a method for reducing reactive oxygen species ROS in the eye of a subject in a cell comprising contacting the cell with the isolated nucleic acids, the rAAVs, or the compositions described herein. In some embodiments the ROS is a peroxide, superoxide, hydroxyl radical, singlet oxygen, or alpha-oxygen. In some embodiments, the cell is a photoreceptor, a neuron, or a ganglion.

In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject inhibits oxidative stress in a subject between 2-fold

and 100-fold (*e.g.*, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 75-fold, 100-fold, *etc.*) compared to a control subject. In some embodiments, administering the isolated nucleic acids, the rAAVs, or the compositions described herein to a subject reduces ROS in a subject between 2-fold and 100-fold (*e.g.*, 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 75-fold, 100-fold, *etc.*) compared to a control subject.

As used herein, the term "treating" refers to the application or administration of a composition (*e.g.,* an isolated nucleic acid or rAAV as described herein) to a subject, who has a disease or disorder associated with prolonged oxidative stress and/or increased levels of OXR1 and/or NCOA7 (including ocular disorders (*e.g.*, retinitis pigmentosa, age-related macular degeneration, retinopathy of prematurity, diabetic retinopathy) and neurodegenerative disorders (*e.g.,* amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Parkinson's disease, Huntington's disease, and lupus)), with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disorder, the symptom of the disease, or the predisposition toward a disease associated with prolonged oxidative stress.

Alleviating a disease associated with prolonged oxidative stress and/or increased levels of OXR1 and/or NCOA7 includes delaying the development or progression of the disease, or reducing disease severity. Alleviating the disease does not necessarily require curative results. As used therein, "delaying" the development of a disease (such as a disease associated with inflammation (e.g., microgliosis), demyelination, and/or death of synaptic neurons) means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. A method that "delays" or alleviates the development of a disease, or delays the onset of the disease, is a method that reduces probability of developing one or more symptoms of the disease in a given time frame and/or reduces extent of the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a statistically significant result.

"Development" or "progression" of a disease means initial manifestations and/or ensuing progression of the disease. Development of the disease can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, development or progression refers to the biological course of the symptoms. "Development" includes

occurrence, recurrence, and onset. As used herein "onset" or "occurrence" of a disease associated with prolonged oxidative stress.

Kits and Related Compositions

The agents described herein may, in some embodiments, be assembled into pharmaceutical or diagnostic or research kits to facilitate their use in therapeutic, diagnostic or research applications. A kit may include one or more containers housing the components of the disclosure and instructions for use. Specifically, such kits may include one or more agents described herein, along with instructions describing the intended application and the proper use of these agents. In certain embodiments agents in a kit may be in a pharmaceutical formulation and dosage suitable for a particular application and for a method of administration of the agents. Kits for research purposes may contain the components in appropriate concentrations or quantities for running various experiments.

The kit may be designed to facilitate use of the methods described herein by researchers and can take many forms. Each of the compositions of the kit, where applicable, may be provided in liquid form (*e.g.,* in solution), or in solid form, (*e.g.,* a dry powder). In certain cases, some of the compositions may be constitutable or otherwise processable (*e.g.,* to an active form), for example, by the addition of a suitable solvent or other species (for example, water or a cell culture medium), which may or may not be provided with the kit. As used herein, “instructions” can define a component of instruction and/or promotion, and typically involve written instructions on or associated with packaging of the disclosure. Instructions also can include any oral or electronic instructions provided in any manner such that a user will clearly recognize that the instructions are to be associated with the kit, for example, audiovisual (*e.g.,* videotape, DVD, *etc.*), Internet, and/or web-based communications, *etc.* The written instructions may be in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which instructions can also reflects approval by the agency of manufacture, use or sale for animal administration.

The kit may contain any one or more of the components described herein in one or more containers. As an example, in one embodiment, the kit may include instructions for mixing one or more components of the kit and/or isolating and mixing a sample and applying to a subject. The kit may include a container housing agents described herein. The agents may be in the form of a liquid, gel or solid (powder). The agents may be prepared sterilely, packaged in syringe and

shipped refrigerated. Alternatively it may be housed in a vial or other container for storage. A second container may have other agents prepared sterilely. Alternatively the kit may include the active agents premixed and shipped in a syringe, vial, tube, or other container. The kit may have one or more or all of the components required to administer the agents to an animal, such as a syringe, topical application devices, or intravenous needle tubing and bag, particularly in the case of the kits for producing specific somatic animal models.

The kit may have a variety of forms, such as a blister pouch, a shrink wrapped pouch, a vacuum sealable pouch, a sealable thermoformed tray, or a similar pouch or tray form, with the accessories loosely packed within the pouch, one or more tubes, containers, a box or a bag. The kit may be sterilized after the accessories are added, thereby allowing the individual accessories in the container to be otherwise unwrapped. The kits can be sterilized using any appropriate sterilization techniques, such as radiation sterilization, heat sterilization, or other sterilization methods known in the art. The kit may also include other components, depending on the specific application, for example, containers, cell media, salts, buffers, reagents, syringes, needles, a fabric, such as gauze, for applying or removing a disinfecting agent, disposable gloves, a support for the agents prior to administration *etc.*

The instructions included within the kit may involve methods for constructing an AAV vector as described herein. In addition, kits of the disclosure may include, instructions, a negative and/or positive control, containers, diluents and buffers for the sample, sample preparation tubes and a printed or electronic table of reference AAV sequence for sequence comparisons.

EXAMPLES

Example 1

The mouse 661W cone photoreceptor cell line was used to produce stable cell lines which overexpress *OXR1* under the control of a strong CB6 promoter. The resistance of the stable cells lines to oxidative stress was examined in the presence of hydrogen peroxide (H_2O_2). Approximately twice the level of H_2O_2 is needed to achieve the same level of cell death in the *OXR1* overexpressing cells as is seen in wild-type 661W cells (FIG. 1). Thus, the 661W cells, which overexpress *OXR1*, may be considerably more resistant to oxidative stress than cells that express *OXR1* at normal levels.

Example 2

The RD1 mouse model (Jackson Laboratories) for retinal degeneration was used to examine the effect of *OXR1* overexpression *in vivo*. This mouse exhibits retinal degeneration as a result of a rapid and progressive retinopathy that is used to model retinitis pigmentosa, a disease that leads to blindness in humans. *OXR1*-expressing AAV gene therapy vectors were produced to test if *OXR1* can protect retinal cells in the RD1 mouse model. DNA encoding the OXR1B1 *OXR1* isoform under the control of the strong CB6 promoter was packaged into AAV8 serotype capsids because of its high affinity for photoreceptor cells.

Subretinal injection of this AAV vector results in infection of about 40-50% of the retinal cells. Normally, the RD1 mouse develops photoreceptor dysfunction and degeneration by about 4 weeks of age. One eye of a cohort of RD1 mice was treated with the AAV-OXR1 vector and when they reached 4 weeks of age, the mice were tested by electroretinography (ERG) to measure visual function, comparing the treated versus untreated eyes.

All of the mice had elevated levels of activity in the treated versus the untreated eye. FIG. 2A shows the average scotopic and photopic wave peaks as determined by ERG. Treated eyes showed a 3.6-fold higher level of peak scotopic a wave activity, a 3.5-fold higher level in the peak scotopic b wave activity, and a 4.8-fold higher level in the peak photopic b wave activity, following subretinal injection, relative to control. The average scotopic b and photopic b wave peak amplitudes were determined by ERG for all treated and control eyes for twelve weeks after subretinal injection (weeks post-injection; 'wpi'). As shown in FIG. 2B and 2C respectively, the scotopic b and photopic b wave peak amplitudes remained elevated in treated eyes for the entire twelve week periods, relative to control eyes.

Subjects suffering from retinitis pigmentosa can lose up to 90% of the cone cells in the fovea of the retina before recognizing the onset of the disease. Thus, the level of ERG activity retention in these initial experiments may confer a substantial benefit to subjects suffering from retinitis pigmentosa.

Materials and Methods

Six isoforms of OXR1 (OXR1A1, OXR1A2, OXR1B1, OXR1B2, OXR1D1, OXR1D2) and two isoforms of NCOA7 (NCOA7-FL and NCOA7-AS) (**Table 1, below, and FIGs. 3-4**) were cloned into an AAV vector comprising a chicken-beta-actin (CBA) promoter.

Table 1. Protein isoforms

Isoform	MW (kDa)	Amino Acids	pI	Base Pairs	Total Exons	Coding Exons
OXR1-A1	97.79	873	5.09	2622	17	15
OXR1-A2	94.57	846	5.00	2541	16	14
OXR1-B1	96.83	866	4.90	2601	15	15
OXR1-B2	93.61	839	4.83	2520	14	14
OXR1-D1	27.64	243	6.88	732	6	6
OXR1-D2	24.42	216	6.51	651	5	5
NCOA7-FL	105.92	942	5.27	2829	16	14
NCOA7-AS	25.22	219	5.60	660	5	5

5

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising a transgene comprising a sequence as set forth in any one of SEQ ID NOs: 8-14 or 32-40 flanked by two adeno-associated virus (AAV) inverted
5 terminal repeats (ITRs).

2. An isolated nucleic acid comprising a transgene encoding a protein having an amino acid sequence as set forth in any one of SEQ ID NOs: 15-22.

10 3. The isolated nucleic acid of claim 1, wherein the transgene encodes a sequence that is at least 70% identical to a nucleotide sequence as set forth in any one of SEQ ID NOs: 8-14 or 32-40.

4. The isolated nucleic acid of claim 2, wherein the transgene encodes a sequence that is at
15 least 70% identical to an amino acid sequence as set forth in any one of SEQ ID NOs: 15-22.

5. The isolated nucleic acid of any one of claims 1-4, wherein the transgene is operably linked to a promoter.

20 6. The isolated nucleic acid of claim 5, wherein the promoter is a tissue-specific promoter or a constitutive promoter, optionally wherein the tissue-specific promoter is an ocular tissue promoter.

7. The isolated nucleic acid of any one of claims 2-6, wherein the transgene is flanked by
25 adeno-associated virus (AAV) inverted terminal repeats (ITR).

8. The isolated nucleic acid of claim 7, wherein at least one AAV ITR lacks a functional terminal resolution site (TRS).

30 9. The isolated nucleic acid of any one of claims 1-8, wherein the AAV ITRs are AAV2 ITRs.

10. The isolated nucleic acid of any one of claims 1-9, wherein the isolated nucleic acid is contained in a vector.

5 11. The isolated nucleic acid of claim 10, wherein the vector is a plasmid or a Baculovirus vector.

12. A recombinant AAV (rAAV) comprising:
the isolated nucleic acid of any one of claims 1-9;
10 and an AAV capsid protein.

13. The rAAV of claim 12, wherein the isolated nucleic acid encodes a protein having a sequence set forth in any one of SEQ ID NOs: 8-14 or 32-40.

15 14. The rAAV of any one of claim 12 or 13, wherein the rAAV is a self-complementary AAV (scAAV).

15. The rAAV of any one of claims 12-14, wherein the AAV capsid protein has a tropism for ocular cells.

20

16. The rAAV of any one of claims 12-15, wherein the capsid protein is an AAV8 capsid protein.

17. A composition comprising the isolated nucleic acid of any one of claims 1-11 or the
25 rAAV of any one of claims 12-16 and a pharmaceutical excipient.

18. A host cell comprising the isolated nucleic acid of any one of claims 1-11 or the rAAV of any one of claims 12-16.

30 19. The host cell of claim 18, wherein the host cell is a bacterial cell, a mammalian cell, or an insect cell.

20. The host cell of claim 19, wherein the mammalian cell is a photoreceptor cell.

21. A method of inhibiting neuronal cell degeneration in a subject comprising administering to the subject the isolated nucleic acid of any one of claims 1-11, the rAAV of any one of claims 12-16, or the composition of claim 17 in an amount effective to inhibit neuronal cell degeneration relative to a subject that has not been administered the rAAV.

22. The method of claim 21, wherein the neuronal cells are photoreceptor cells.

23. The method of claim 21 or 22, wherein the subject has or is suspected of having a disease associated with neuronal cell degeneration, optionally wherein the disease is associated with degeneration of ocular cells.

24. The method of any one of claims 21-23, wherein the rAAV is administered to the subject by intraocular injection, subretinal injection, intraneural injection, intrarenal injection, intravenous injection, intramuscular injection, or infusion.

25. The method of any one of claims 21-24, wherein neuronal cell degeneration is inhibited by between 2-fold and 100-fold following the administration.

26. The method of any one of claims 23-25, wherein the degenerative disease is retinitis pigmentosa, age-related macular degeneration, retinopathy of prematurity, or diabetic retinopathy.

27. A method for treating a disease or disorder associated with photoreceptor cell degeneration in a subject comprising:

administering to the subject the isolated nucleic acid of any one of claims 1-11, the rAAV of any one of claims 12-16, or the composition of claim 17.

28. The method of claim 27, wherein the disease is retinitis pigmentosa, age-related macular degeneration, retinopathy of prematurity, or diabetic retinopathy.

29. The method of claim 27 or 28, further comprising measuring photoreceptor cell activity by electroretinography (ERG).

30. The method of any one of claims 27-29, wherein after the administration the subject has between 3.5-fold and 100-fold higher peak scotopic a wave activity relative to an untreated subject.

31. The method of any one of claims 27-30, wherein after the administration the subject has between 3.5-fold higher and 100-fold higher peak scotopic b wave activity relative to an untreated subject.

32. The method of any one of claims 27-31, wherein after the administration the subject has between 4.8-fold and 100-fold higher level in the peak photopic b wave activity relative to an untreated subject.

33. The method of any one of claims 27-32, wherein the administration is intraocular injection, subretinal injection, intraneural injection, intrarenal injection, intravenous injection, intramuscular injection, or infusion.

34. The method of any one of claims 27-33, wherein the isolated nucleic acid, the rAAV, or the composition transduces neuronal cells.

35. The method of claim 34, wherein the isolated nucleic acid, the rAAV, or the composition transduces retinal cells.

36. The method of claim 34, wherein the isolated nucleic acid, the rAAV, or the composition transduces photoreceptor cells.

37. A method for inhibiting oxidative stress in a cell comprising contacting the cell with the isolated nucleic acid of any one of claims 1-11, the rAAV of any one of claims 12-16, or the composition of claim 17 in an amount sufficient to reduce reactive oxygen species (ROS) in the cell.

38. The method of claim 37, wherein the cell is a neuronal cell, a photoreceptor cell, a pigmented retinal epithelial cell, or a glial cell.

5 39. The method of claim 37 or 38, wherein the cell is in a subject.

40. The method of claim 39, wherein the subject has a disease associated with neuronal degeneration.

10 41. The method of claim 40, wherein the subject has a disease associated with ocular cell degeneration

42. The method of claim 40 or 41, wherein the disease is retinitis pigmentosa, age-related macular degeneration, retinopathy of prematurity, or diabetic retinopathy.

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43. A kit comprising a container enclosing the isolated nucleic acid of any one of claims 1-11, the rAAV of any one of claims 12-16, or the composition of claim 17.

44. The kit of claim 43, wherein the container is a syringe.

20

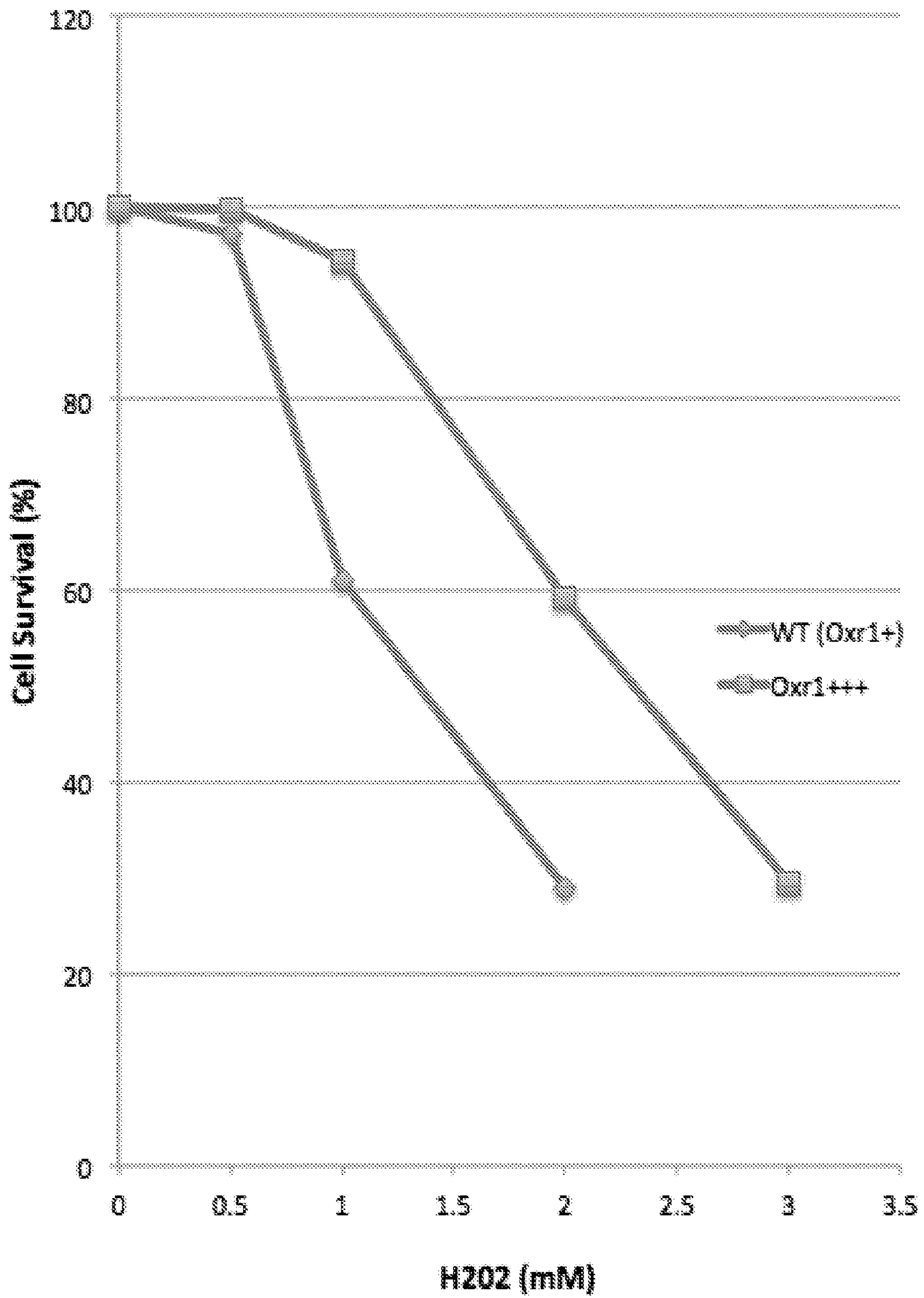

FIG. 1

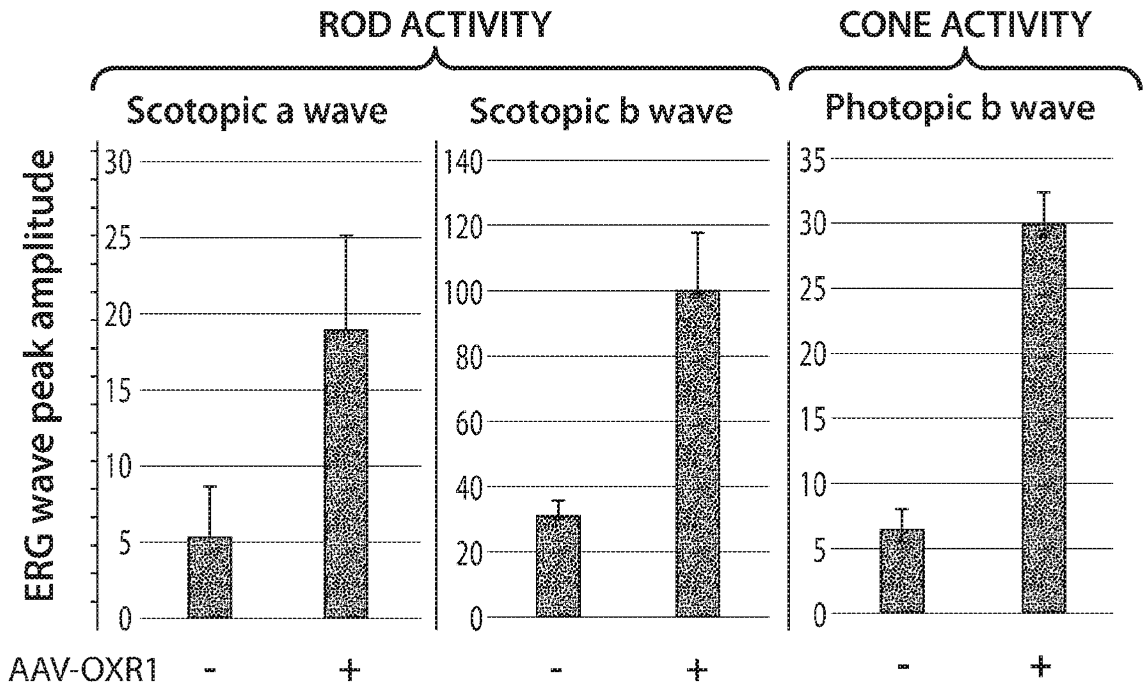

FIG. 2A

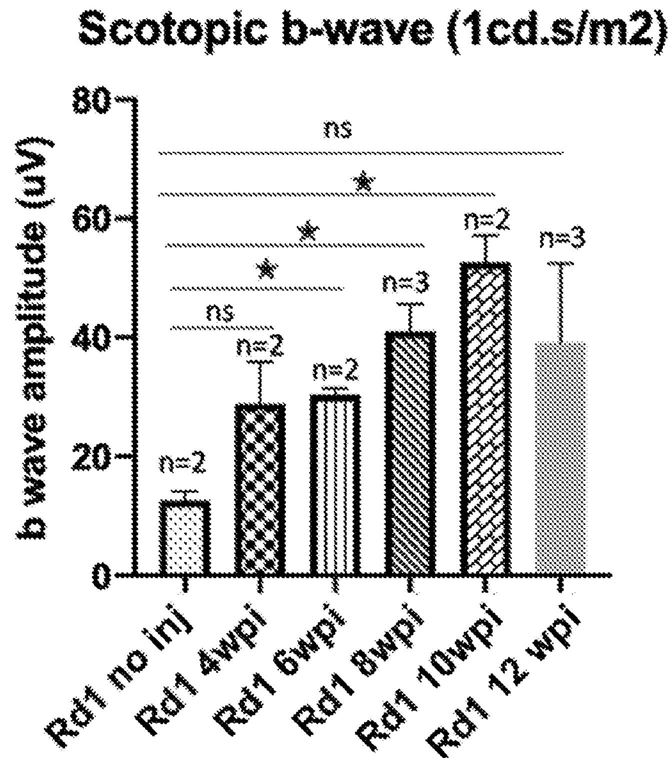

FIG. 2B

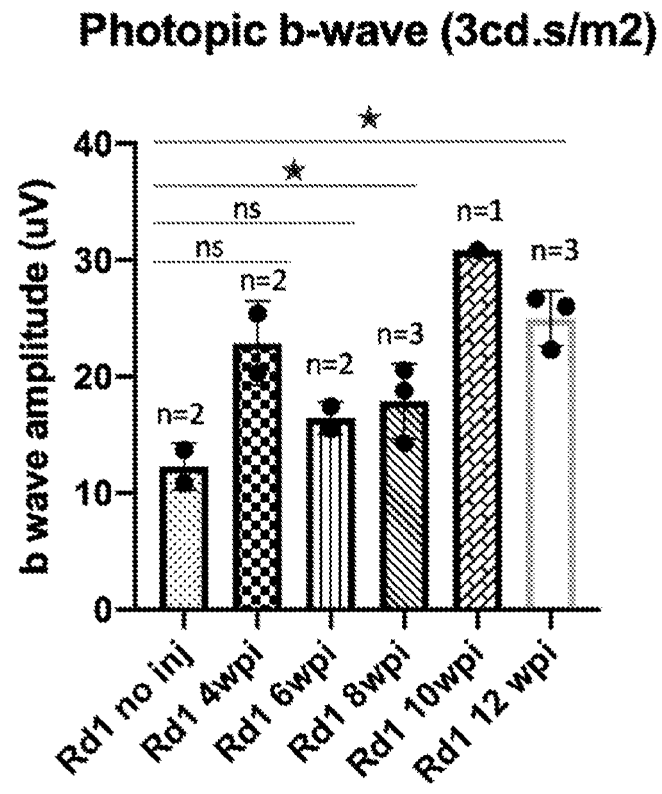

FIG. 2C

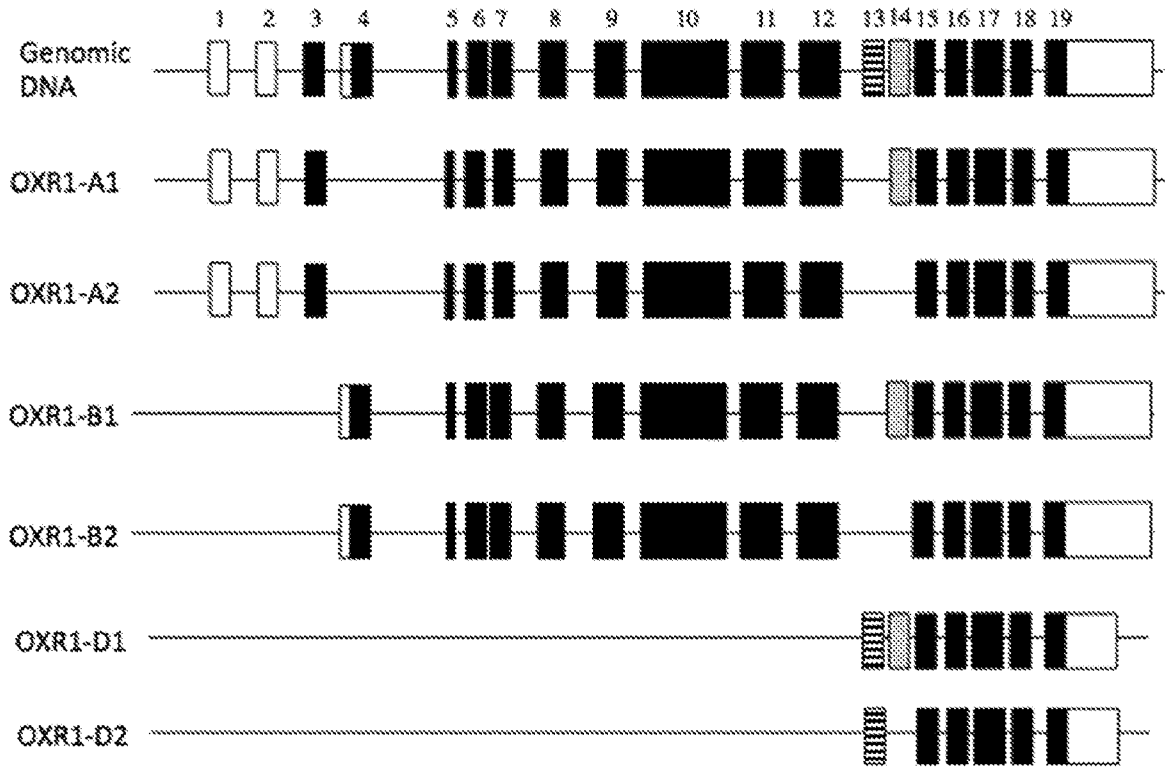

FIG. 3

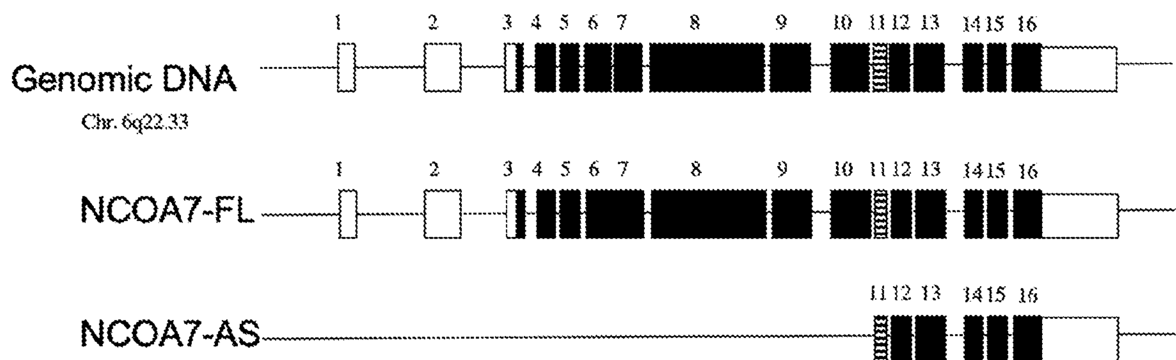

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/019234

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K48/00 C07K14/47
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01K A61K C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] 27 January 2010 (2010-01-27), "Homo sapiens mRNA for oxidation resistance 1 isoform A (OXR1 gene)", XP002798998, retrieved from EBI accession no. EM_STD:FN650108 Database accession no. FN650108 sequence -----	2,4-6, 10,11, 18,19
X	WO 2013/151665 A2 (MODERNA THERAPEUTICS [US]) 10 October 2013 (2013-10-10) sequences 21295, 21296, 21290, 21289, 21292 -----	2,4-6, 10,11, 18,19
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 May 2020	22/07/2020

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Fax: (+31-70) 340-3016

Authorized officer
Lewis, Birgit

7

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LIJIAN YU ET AL: "Induction of a Unique Isoform of the NCOA7 Oxidation Resistance Gene by Interferon [beta]-1b", JOURNAL OF INTERFERON AND CYTOKINE RESEARCH., vol. 35, no. 3, 1 March 2015 (2015-03-01), pages 186-199, XP055694892, US ISSN: 1079-9907, DOI: 10.1089/jir.2014.0115 the whole document -----	1-44
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A	PETER L. OLIVER ET AL: "Oxr1 Is Essential for Protection against Oxidative Stress-Induced Neurodegeneration", PLOS GENETICS, vol. 7, no. 10, 20 October 2011 (2011-10-20), page e1002338, XP055427887, DOI: 10.1371/journal.pgen.1002338 the whole document -----	1,3,7-9, 12-17, 20-44

7

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Information on patent family members

International application No
PCT/US2020/019234

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Information on patent family members

International application No
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/019234

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-44(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-44(partially)

   Subject-matter relating to a transgene encoding OXR1 (SEQ ID NOs: 8-13 and 15-20)

   ---

2. claims: 1-44(partially)

   Subject-matter relating to a transgene encoding NCOA7 (SEQ ID NOs: 14,32-40 and 21,22)