

Update on Gene Therapy for the Treatment of Retinal Diseases

Gene therapy can correct underlying genetic defects that cause disease, potentially with lifelong clinical benefits based on a single therapeutic administration.

BY MARK E. PENNESI, MD, PhD

Genes enable production of proteins that perform a vast array of functions within all living organisms. Many diseases have a genetic aspect whereby a mutated gene is passed down from generation to generation. Mutated genes can cause production of abnormal proteins or disable the production of a protein completely, either of which can cause disease.

Gene replacement therapy involves the introduction of a functional copy of the gene into a patient's own cells using a delivery system most commonly based on a viral vector to treat the genetic defect. By correcting the underlying genetic defect that is the cause of disease, gene therapy can provide transformative disease-modifying effects, with the potential for lifelong clinical benefits based on a single therapeutic administration.

DELIVERING GENES WITH ADENO-ASSOCIATED VIRUSES

Most gene therapy uses viral vectors to deliver therapeutic genes into cells affected by disease. Viral vectors have been optimized for this purpose by removing pathogenic elements and severely impairing the virus' ability to replicate. Adeno-associated virus (AAV) vectors are particularly well suited for treating retinal diseases and have advantages over other viral vectors, such as adenovirus, herpes virus, and lentivirus. AAV is a small, simple, nonenveloped virus with only two native genes. This makes the virus straightforward to work with from a vector-engineering standpoint. AAV vectors have

the capacity to carry therapeutic gene sequences up to 4000 base pairs in length.¹ Because more than 90% of human genes have coding sequences less than 3000 base pairs in length, a wide variety of genetic diseases could be treatable with AAV-mediated gene therapy.

AAV vectors have to date been safe for use in human gene therapy. AAV has never been linked to human disease, unlike most other viruses used as gene delivery vectors—such as lentivirus, adenovirus, and herpes virus. AAV elicits only a mild immune response, reducing the risk of adverse inflammatory reactions. AAV vectors have no viral genes remaining, virtually eliminating the possibility that any viral genes will cause an adverse event.¹ AAV vectors have been used in more than 100 human clinical trials with no serious adverse events traced to the use of AAV as the gene delivery vector. Recombinant AAV can now be produced at a commercial scale in adherence with current good manufacturing practice for use in clinical trials and for future marketed products. As many as 2.4×10^{14} vector copies per liter have been produced with the latest advance in large-scale viral vector production technology.² The purification process eliminates all but a trace amount of the raw materials used during manufacture, in many cases below the level of detection of the assays.³

UPDATE ON GENE THERAPIES FOR RETINAL DISEASES

Leber Congenital Amaurosis (LCA)

The LCA gene therapy program is the most extensive to date. LCA is a form of early-onset, inherited retinal

degeneration caused by mutations in any of at least 16 genes involved in retinal function. It leads to blindness at birth or in early childhood or adolescence. One form of LCA is caused by mutations in the RPE65 gene, which encodes an enzyme that replenishes visual pigment and is critical for normal phototransduction. The safety of a single subretinal injection of AAV vector expressing human RPE65 has been supported in multiple phase 1 clinical trials. No serious adverse events or clinically significant immune responses were reported in the trials, with 3 years the longest reported follow-up.⁴⁻⁶ The safety and efficacy of RPE65 gene therapy for LCA continues to be evaluated in ongoing clinical trials including a phase 3, randomized, open-label controlled study (NCT00999609) with primary efficacy results expected in mid-2015.

Achromatopsia

Achromatopsia is an inherited retinal disease characterized by a lack of cone photoreceptor function. Individuals with this condition have markedly reduced visual acuity, nystagmus, photophobia, and complete loss of color discrimination. Achromatopsia can be caused by mutations in any of at least 5 genes that are required for normal cone photoreceptor function. The most common causes are mutations in the CNGB3 gene (about half of all cases) or CNGA3 gene (about one-fourth of all cases). These genes encode proteins that combine to form a channel in the photoreceptor membrane that is required for phototransduction. Achromatopsia occurs in two breeds of dogs due to CNGB3 mutations that either produce an abnormal protein or prevent production of the protein. Both breeds have clinical characteristics similar to humans with achromatopsia. Subretinal injection of an AAV vector expressing human CNGB3 restored cone function in dogs with either mutation and improved the dogs' ability to navigate mazes under bright light conditions.⁷ Preclinical evaluation in large mammals is ongoing (Figure). A prospective 3-year observational study to characterize the clinical features of patients with achromatopsia caused by mutations in the CNGB3 gene (NCT01846052) is currently recruiting patients, with completion estimated for mid-2017. Gene therapy programs to develop treatment for CNGA3 mutations are also under way.

X-linked Retinoschisis (XLRs)

XLRs is an inherited retinal disease caused by mutations in the RS1 gene, which encodes the retinoschisin protein. Retinoschisin is expressed and secreted primarily from photoreceptor and bipolar cells and binds strongly and specifically to the surface of many cells in the retina. Mutated forms of retinoschisin are unable to bind properly, resulting in schisis, or splitting, of all layers of the retina, primarily in

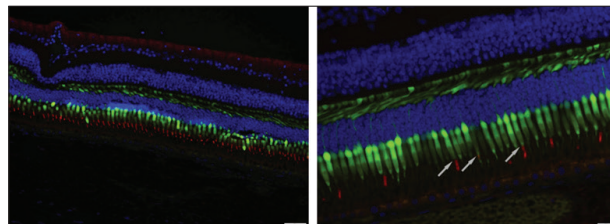


Figure. Efficient transduction of long- (L), medium- (M), and short-wavelength (S) cones with AAV vector expressing genes under control of the PR1.7 photoreceptor-specific promoter. Panels show cross-sections of retina from a nonhuman primate after treatment with an AAV vector expressing green fluorescent protein (GFP) under control of the PR1.7 promoter. The slide in the left panel has been stained for GFP (green) and L- and M-cone outer segments (red). The slide on the right panel was stained for GFP (green) and S-cone outer segments (red). Cell nuclei were labeled with DAPI (blue). The alignment of green and red fluorescence in each panel demonstrates efficient transduction of all cone types when using an AAV vector expressing genes with the PR1.7 promoter. Bar in left panel is 50 μ m. Bar in right panel is 20 μ m.

the macula and periphery.

An XLRs knockout mouse model has been developed with clinical features similar to those of humans with XLRs, including reduced visual acuity, schisis cavities detected by optical coherence tomography (OCT), and abnormal electroretinogram (ERG) response. Subretinal or intravitreal injection of an AAV vector expressing human RS1 in these knockout mice restored organization of the retinal cell layers and improved visual function as measured by increased ERG b-wave responses.^{8,9} Preclinical evaluation in mice and large mammals is ongoing. Two phase 1/2 clinical trials are planned to evaluate the safety and tolerability of an intravitreal injection of AAV vector expressing RS1 in patients with XLRs. One trial (NCT02317887) is currently recruiting patients and the other is scheduled to start recruitment in the first half of 2015.

X-linked Retinitis Pigmentosa (XLRP)

Retinitis pigmentosa is an inherited retinal dystrophy associated with progressive loss of vision. It is commonly observed in boys and young men who first experience night blindness, then constricted peripheral vision, and, eventually, legal blindness. In the United States, about 15% of retinitis pigmentosa cases are X-linked. Ninety percent of XLRP cases are caused by mutations in the RPGR gene, which encodes a photoreceptor ciliary protein. A preclinical study in a dog model of XLRP caused by mutations in the RPGR gene demonstrated a delay in the rate of disease progression in eyes that received a subretinal injection of AAV vector expressing RPGR.¹⁰ Preclinical evaluation is ongoing.

Stargardt Disease

Stargardt disease is an inherited retinal dystrophy characterized by photoreceptor degeneration in the macula and the accumulation of yellowish perifoveal flecks. Individuals with this condition often present with reduced visual acuity in late childhood. Most cases of Stargardt disease are caused by mutations in the ABCA4 gene, which encodes a protein transporter required to clear visual cycle products that accumulate in photoreceptors after photoexcitation.¹¹ Patients are currently being enrolled in a phase 1/2 trial that will evaluate the safety and tolerability of a lentiviral vector, based on an equine infectious anemia virus (EIAV), expressing the ABCA4 gene in patients with Stargardt disease (NCT01367444).

Usher Syndrome Type 1B

Usher syndrome is a form of retinitis pigmentosa that causes deaf-blindness. The most severe clinical subtype (type 1) is associated with profound congenital deafness and early-onset photoreceptor loss. About half of Usher type 1 cases are caused by mutations in the MYO7A gene, and these are subclassified as type 1B. Subretinal injection of lentiviral vector (EIAV) expressing the MYO7A gene was safe and well tolerated in nonhuman primates.¹² Patients are currently being enrolled in a phase 1/2 trial that will evaluate the safety and tolerability of lentiviral vector (EIAV) expressing the MYO7A gene in patients with Usher syndrome type 1B (NCT01505062).

Choroideremia

Choroideremia is an inherited degenerative disease of the retinal pigment epithelium, choroid, and retina associated with progressive vision loss and eventual blindness. Choroideremia is X-linked and caused by mutations in the CHM gene, which encodes Rab escort protein-1 (REP-1). An interim analysis of an ongoing phase 1/2 clinical trial (NCT01461213) reported promising visual function and retinal sensitivity results 6 months after subretinal injection of an AAV vector expressing the CHM gene.¹³ Recruitment recently began for a second phase 1/2 trial (NCT02341807).

Leber Hereditary Optic Neuropathy (LHON)

LHON is a maternally inherited disorder characterized by retinal ganglion cell loss, optic nerve atrophy, and central vision loss. Nearly all cases of LHON are caused by mutations in any one of three mitochondrial genes (ND1, ND4, and ND6). These genes encode proteins that form part of a complex that is required to support ATP synthesis and reduce levels of oxidative stress. About half

of LHON cases are caused by the same single base pair substitution in the ND4 gene.^{14,15} Recruitment is ongoing for two phase 1 clinical trials that will evaluate safety and tolerability of AAV vectors expressing the ND4 gene in LHON patients with ND4 mutations (NCT02161380, NCT02064569).

Blue Cone Monochromacy (BCM)

BCM is an inherited color vision deficiency characterized by lack of functional long-wavelength (L) and medium-wavelength (M) cone photoreceptors but generally normal function of short-wavelength (S) cone photoreceptors. BCM can be caused by mutations in the part of the X chromosome called the locus control region, which regulates expression of the L and M opsin genes.¹⁶ The use of gene therapy to bestow trichromatic color vision was demonstrated in a preclinical study of dichromatic nonhuman primates that were missing the L opsin gene. Trichromatic vision behavior was observed after subretinal injection of an AAV vector expressing the human L opsin gene.¹⁷ Preclinical evaluations of gene therapy for color vision deficiencies, including BCM, are ongoing.

Wet Age-Related Macular Degeneration (AMD)

Wet AMD is a major cause of blindness and visual impairment in older adults. Wet AMD is associated with neovascularization and macular edema that is stimulated by VEGF. Current standard of care for wet AMD is intravitreal injection of anti-VEGF agents, which must be repeated monthly or bimonthly over a prolonged period.

An emerging wet AMD gene therapy approach uses an AAV vector to deliver a gene called sFLT01 into retinal cells. The sFLT01 gene is an engineered version of the VEGF receptor that binds and inhibits VEGF ligand.¹⁸ Gene therapy with sFLT01 has been evaluated in preclinical studies using accepted animal models of retinal neovascular diseases. Intravitreal injection of AAV vector expressing the sFLT01 gene markedly reduced retinal neovascularization and vascular leakage in a nonhuman primate model.¹⁹ A related study showed that intravitreal injection of AAV vector expressing sFLT01 was well tolerated and maintained long-term expression in nonhuman primates after 12 months of follow-up.²⁰ The safety and tolerability of sFLT01 gene therapy for wet AMD is currently under investigation in an active phase 1 clinical trial (NCT01024998).

Other AMD gene therapy programs include an active phase 1/2 trial evaluating the safety of a subretinal injection of AAV vector expressing the naturally occurring VEGF decoy receptor sFlt1 (NCT01494805) and an active phase 1 trial evaluating the safety of a subretinal

injection of lentiviral vector (EIAV) expressing the antiangiogenic proteins endostatin and angiostatin (NCT01301443).

POTENTIAL CHALLENGES AND FUTURE OUTLOOK

The promise of gene therapy has evolved over the past decade, with a growing body of preclinical and clinical data providing evidence of safety and efficacy in a variety of retinal disease areas. By correcting an underlying defect, gene therapy can provide transformative disease-modifying effects, potentially with lifelong clinical benefits based on a single therapeutic administration. The results of prospective, long-term studies will provide greater insight into the extended safety and efficacy of single treatments for each disorder.

AAV vectors have the capacity to carry therapeutic gene sequences of up to 4000 base pairs in length. While more than 90% of human genes have coding sequences less than 3000 base pairs in length, a few therapeutic coding sequences exceed the AAV carrying capacity, including those for Stargardt disease and Usher syndrome type 1B. Approaches that have been used to overcome this obstacle include the use of viral vectors with the capacity to carry gene sequences that are longer than 4000 base pairs, or gene therapy techniques that inject two AAV vectors at once, each expressing one half of the complete sequence and engineered to reconstitute by splicing after transfer into a patient's cells.²¹

Each retinal disorder has a unique disease course, and natural history data are critical to determine optimal timing for intervention with gene therapy. Natural history data are published for achromatopsia, choroideremia, Stargardt disease, and XLRP caused by mutations in the RPGR gene.^{11,22,23} Natural history studies are planned or in progress for achromatopsia, XLRs, Stargardt disease, and Usher syndrome.

An exciting advance in the treatment of late stage retinal degeneration is an approach that bypasses photoreceptors by delivering a light-sensitive protein to neurons in the retina. One such light-sensitive protein is channelrhodopsin 2, or ChR2, a protein that controls photosynthesis in green algae. When ChR2 is inserted into a neuron and the neuron is stimulated by light, the neuron is activated and can transmit a signal to the visual cortex. This technique, called optogenetics, is being applied to the design of therapeutic genes that can be expressed by AAV vectors for the treatment of advanced retinal degeneration.²⁴

While additional study is needed to address current challenges and pursue potential advances, preclinical and clinical findings to date provide compelling support for

continued investment in the development of AAV-based gene therapies for the treatment of patients with retinal diseases. ■

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