

OCULAR GENE THERAPY TRIALS ADVANCE

Results of the first phase 3 trial were announced.

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"We used to think that our fate was in our stars, but now we know that, in large measure, our fate is in our genes."

—James Watson¹

Genetic alterations are known to be responsible for numerous diseases, and so it follows logically that the best cures for these diseases

might lie in correcting genetic anomalies by the process of gene therapy. This approach involves the introduction of genes into existing cells in attempts to prevent or cure a wide range of diseases previously thought to be incurable.¹

Posterior segment disorders are challenging to treat, and current therapies have numerous shortcomings. Many are invasive, run the risk of complications, offer only short-term relief from symptoms, or are unable to directly treat vision loss. Ocular gene therapy has generated interest due to the potential of circumventing these challenges and delivering lasting cures for a variety of diseases of the posterior segment.

This installment of the *Innovations in Retina* column details several ocular diseases that could potentially benefit from gene therapy and describes successful clinical research in the treatment of Leber congenital amaurosis (LCA) that could have potential implications for the field in general.



AT A GLANCE

- Gene therapy offers an opportunity to manage difficult-to-treat diseases based on genetic mutation.
- Researchers are currently investigating gene therapy options for diseases such as retinitis pigmentosa, achromatopsia, juvenile retinoschisis, and choroideremia.
- Results from trials using gene therapy for treatment of LCA have shown promise, but also variability.

AAV FACILITATES GENE DELIVERY

The nonpathogenic adeno-associated virus (AAV) has to date been a safe and effective vector for gene delivery. The recombinant AAV (rAAV) vector has demonstrated increased specificity and efficiency in ocular AAV-mediated gene therapy interventions. Recombinant AAV2 (rAAV2) vectors used for gene therapy are derived from the wild-type virus by deleting the entire viral coding region and replacing it with the reporter or therapeutic transgene. Combined AAV serotypes have also been developed. This diversity of serotypes may lead to more specific and more efficient transduction. However, successful and efficient transfection of particular cell types in the eye still depends on other factors, such as the AAV titer, the site of injection, the amount of passenger DNA, and the specific gene promoters where transcription initiation takes place.²

Retinal degeneration and visual function loss in genetically engineered and naturally occurring animal models have been characterized by a variety of methods, and initial success has been observed in the treatment of some retinal disorders.

rAAV-MEDIATED TREATMENT

In recent years, a number of retinal and optic nerve-related conditions have been identified with causative gene mutations that could be amenable to rAAV-mediated gene therapy. Researchers have been developing these therapeutic modalities in the clinic, and, while most have encountered challenges, there has been encouraging success that could revolutionize retinal medicine.

LCA is an autosomal recessive ocular disorder that appears at birth and is characterized by sluggish or absent pupillary response due to mutations in the RPE65, LRAT, RDH12, or RPEGRIIP genes. Gene therapy has been successful against mutations in LRAT and RPE65, where it has improved vision by restoring photoreceptor function, as described later in this article.³

The autosomal recessive form of retinitis pigmentosa, which manifests as early-onset retinal dystrophy and tunnel vision preceded by night blindness, is characterized by mutations in genes encoding the phototransduction proteins and those encoding the regulatory network proteins in the

outer segment of the photoreceptors PDE6B and MERTK, respectively. rAAV5-mediated gene replacement of the hypomorphic PDE6B allele, injected subretinally in an rd10 mouse model with partial PDE6B deficiency, demonstrated prolonged photoreceptor survival and improved vision.³

Achromatopsia is another autosomal recessive condition, marked by early-onset retinal dystrophy, lack of cone function resulting in color blindness, reduced central vision, and photophobia. It is caused by mutations in GNAT2, which encodes a component of the cone phototransduction cascade, and in CNGB3, which encodes the β -subunit of the cyclic nucleotide-gated cation channel in cones. Success of gene therapy to improve cone function may depend on the age at which patients receive treatment, but AAV2/5-mediated gene replacement has been successful in a dog model.³

Juvenile retinoschisis is an X-linked recessive condition with early onset of retinal disease in which the retina separates into several layers and may detach. An inherited early-onset retinal degenerative disease that is the leading cause of juvenile macular degeneration in males, it is caused by mutation in the RS1 gene that encodes retinoschisin, a protein integral to the retina for cellular adhesion and tissue stability. AAV5-mediated gene therapy was found to improve retinal function in a mouse model of the disease.³

Multiple sclerosis may have a multifactorial inheritance pattern, resulting in optic neuritis that causes loss of visual function after multiple neuritis episodes. Therapy mediated by the free radical scavenger AAV-catalase targets oligodendrocytes to suppress their demyelination.³

Red-green color blindness is an X-linked recessive condition that presents with cone dystrophy, resulting in the inability to distinguish red from green. It is caused by congenital absence of the L-opsin gene that encodes L-photopigment. This defect has been corrected in adult male squirrel monkeys through rAAV2/5-mediated replacement of the L-opsin gene.³

Wet age-related macular degeneration is characterized by choroidal neovascularization (CNV), in which blood and other fluids leak into the macula from abnormal new vessels in the choroid. Treatment with rAAV-PEDF transgene resulted in a reduction in the development of CNV and regression of already developed CNV in a mouse model.³

A number of companies have jumped on the bandwagon and are investigating the potential of gene therapy to treat or cure conditions of the posterior segment. NightstaRx is developing an AAV2-REP1 product that could potentially treat choroideremia, an X-linked recessive degenerative retinal disorder, with a single injection and relatively few risks to patients. In a clinical trial, after 6 months of treatment, six of 12 patients showed vision improvement in dim light, and two patients with impaired visual acuity were able to read more lines on an eye chart.⁴

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AGTC has several ongoing ophthalmology development programs. In partnership with Biogen, AGTC is developing its lead rAAV gene therapy product targeting the RS1 gene for the treatment of X-linked juvenile retinoschisis.⁵

Oxford BioMedica reported encouraging results from a phase 1 trial of its antiangiogenic product RetinoStat for the treatment of wet AMD. The trial met its primary endpoints of safety and tolerability, and patients also showed signs of clinical benefit, with visual acuity stabilization and a reduction in vascular leakage.⁶⁻⁸ The product uses the company's LentiVector platform technology to deliver two genes encoding the antiangiogenic proteins endostatin and angiostatin directly to the retina in a single injection.⁸

Phase 1/2 trials using the LentiVector platform are ongoing for the treatment of Stargardt disease, which is characterized by macular degeneration in juveniles, and Usher syndrome type 1B, characterized by adolescent-onset retinitis pigmentosa combined with a congenital hearing defect.⁶ In these trials, Oxford BioMedica's technology is used to deliver to retinal cells, in a single administration, a corrected version of either the ABCR gene for the treatment of Stargardt disease or the MYO7A gene for treatment of Usher syndrome type 1B.^{9,10}

GENE THERAPY FOR LCA

Although most investigations of ocular gene therapy to date have been in preclinical studies and early clinical development, Spark Therapeutics has reported clinical success in the treatment of LCA.

A number of LCA-RPE65 gene therapy trials are under way. The first three trials, initiated in 2007, used a single subretinal injection of an rAAV2 vector to deliver a human RPE65 cDNA gene to target retinal pigment epithelium (RPE) cells. Differences among the three trials included surgical protocols, DNA promoter types, patient characteristics, some of the vision testing methods used, and vector preparation and volumes.²

Gene therapy for patients with LCA due to RPE65 mutations appeared to be safe via subretinal injection at

the doses tested in those trials, with no serious adverse events, no systemic toxicity, and minimal or absent immune response. A macular hole occurred in one patient in one trial, and thinning of the fovea was reported in one patient in another trial. Investigators in these trials reported that visual function was improved for many but not all of the 18 patients. Efficacy results included improved retinal sensitivity, improved pupillary response, improved visual acuity, and reduced rapid involuntary eye movements.² Results also included the development of pseudo-fovea in one patient, and further studies have suggested that this indicates the need for careful consideration of treatment zones for future gene therapy trials.^{2,11}

Although electroretinography response was generally not improved, possibly due to the small treatment area used, some patients showed improved mobility and were able to better navigate an obstacle course using their treated eye, as compared with their untreated eye and their baseline abilities recorded before treatment. Although these results were encouraging, the data from these studies have shown variability. Comparing results across the trials has been limited by differences in gene therapy product profile, administration or injection site, patient selection, and endpoint measurement. The small number of patients in these phase 1 and 2 studies has further limited the ability to compare datasets.^{2,12}

PHASE 3 RESULTS REPORTED

Since the LCA studies discussed above were initiated, other academic groups have been developing gene therapy programs for the treatment of LCA type 2, but the Spark Therapeutics technology, licensed from the Children's Hospital of Pennsylvania, is the furthest advanced, and a phase 3 study was recently completed.¹²

In this first randomized, controlled, phase 3 gene therapy study for an ocular genetic disease, Spark Therapeutics recently announced positive results using SPK-RPE65 to treat LCA2 and other RPE65-mediated inherited retinal dystrophies.¹³ Subjects in the intervention group achieved statistically significant improvement over control subjects in both functional vision and light sensitivity.

The primary endpoint in this study was change in mobility test score 1 year after therapy. During the mobility test, patients were assessed on their ability to navigate an obstacle course or maze under seven light intensity levels ranging from 1 (equivalent to a moonless summer night) to 400 (equivalent to an office setting). The maximum light intensity level at which they could not successfully traverse the maze was recorded.¹²

The trial of 31 subjects with confirmed RPE65 gene mutations met the primary endpoint. It also met two of three secondary endpoints: full-field light sensitivity threshold testing and the first eye mobility test change score. The third secondary endpoint of visual acuity change was not achieved,

but a positive trend was seen. There were no drug-related serious adverse events observed and no deleterious immune responses in either this phase 3 trial or in earlier phase 1 studies.^{14,15} Data from the phase 1 cohort showed that the improved mobility and light sensitivity effects were rapid, sustained, and durable even 3 years after therapy.¹⁶

The company described these results as a "watershed moment in the long-time pursuit of innovative gene therapy solutions for a range of blinding retinal degenerative diseases."¹³

THE FUTURE

Success in these initial trials has spearheaded a wave of interest in ophthalmology gene therapy programs, as there are many genetic disorders that affect a variety of retinal tissues. Besides multiple trials on rAAV2-RPE65 injection for RPE65-mediated inherited retinal dystrophies (by Spark Therapeutics, QLT, AGTC, Hadassah Medical Organization, Nantes University Hospital, Scheie Eye Institute, and University College London), trials are planned or ongoing for the treatment of choroideremia using rAAV2-REP1 (Spark Therapeutics, University of Oxford), and for Leber hereditary optic neuropathy using rAAV2/2-ND4 intervention (GenSight Biologics). ■

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- financial interest: none acknowledged