Emerging Gene Therapy Treatments for Inherited Retinal Diseases

by Benjamin Bakall, MD, PhD; and Kendra A. Klein, MD

Inherited retinal dystrophies (IRDs) are a group of severe, progressive, blinding disorders that have long been lacking effective treatment. The successful identification of the causative genes for retinal dystrophies and emerging gene therapy treatment trials have increased the treatment potential for retinal dystrophies.

The recent U.S. Food and Drug Administration approval of the first gene therapy for retinal dystrophy caused by mutations in the RPE65 gene in December 2017 brings retinal disease to the forefront of human gene therapy. This will stimulate the development of new treatment trials for retinal dystrophies and may facilitate the approval process in the near future.

There are several recently completed and currently ongoing gene therapy treatment trials for different IRDs registered in the database clinicaltrials.gov. The specific treatment is dependent on the molecular disease mechanism. For recessive diseases with loss of function, adding the normal gene is a feasible strategy, but for dominant disorders with a dominant negative effect, the overexpression of a normal gene may not be sufficient, and a combined approach with gene suppression and gene replacement may be required.

The most common approach for retinal gene therapy is delivering the normal gene to the retinal cells using a vector, a modified virus engineered to not proliferate or cause structural damage. The gene therapy trials for IRDs mainly utilize the vector adeno-associated virus (AAV) with high retinal affinity and tolerability. Most retinal dystrophy genes are relatively small and can easily be inserted in AAV.\(^1\) The current trials for Stargardt disease (ABCA4) and Usher syndrome type I (MYO7A) use the equine infectious anemia virus (EIAV) vector. The vector is injected into the subretinal space after surgical vitrectomy for all current viral based retinal gene therapy trials except for the treatment of X-linked juvenile retinoschisis, where the vector is injected into the vitreous cavity to decrease the risk for retinal detachment. IRDs caused by alternative splicing can be treated...
by allele-specific oligonucleotides (AONs) that normalize expression by intravitreal injection of RNA molecules, a strategy utilized in patients with Leber congenital amaurosis (LCA) caused by the CEP290 gene. Optogenetics is a disease gene-independent technology for converting the remaining retinal neurons to photosensitive cells by expression of bacterial rhodopsin genes. The recently completed and currently ongoing gene therapy trials for IRDs are listed in the Table and reviewed below.

**LCA, RETINITIS PIGMENTOSA: RPE65**

Individuals affected by mutations in the RPE65 gene have early onset retinal dystrophy, in most cases consistent with LCA (Figure 1), and some have features more consistent with retinitis pigmentosa. The RPE65 gene encodes a key enzyme in the visual cycle that catalyzes the isomerization of 11-trans-retinyl esters into the chromophore 11-cis-retinal.

After several years of intense research, including successful gene therapy on small and larger animals, the first human gene therapy trials started in 2007. Early phase RPE65 gene therapy trials have been conducted at multiple locations with variable results.\(^4\)\(^-\)\(^10\)

The successful efficacy of the outcome for the phase 3 trial can be attributed to the modified promoter for increased gene expression and purification of the vector to reduce empty capsids. A major challenge in developing the phase 3 clinical trial was selecting relevant outcomes in this rod photoreceptor disease. Standard outcomes, including visual acuity and visual field testing, relied upon in traditional ophthalmology clinical trials depend on the cone photoreceptor function. A novel standardized multiluminance mobility test was developed for testing ambulatory vision in different levels of light encountered in daily life.\(^11\)

The phase 3 trial sponsored by Spark Therapeutics was completed in 2015 and showed a significant and clinically meaningful improved ability to complete the mobility test at increasingly dimmer levels of light. In addition, a modified dark adaptation test, the full-field sensitivity threshold (FST), was significantly improved with the treatment. There was stable improvement in the published study at 2 years,\(^12\) and an updated conference report showed durability at 3
years. Anecdotal patient stories included life-changing experiences after the treatment with ability to see stars at night, recognize faces of family members, and ambulate without a white cane.

**CHOROIDEREMIA**

Mutations in the \textit{CHM} gene, encoding for the Rab escort protein-1 (REP1), cause X-linked choroideremia, a progressive retinopathy characterized by night blindness, peripheral visual field constriction, and decreased central vision in affected males (Figure 2). The initial phase 1 / 2 trial in the U.K. showed improved visual acuity in two of the six treated subjects. There are currently several efforts in the U.S. and U.K. recruiting patients for gene therapy treatment trials.

**X-LINKED RETINITIS PIGMENTOSA**

The most common cause for X-linked retinitis pigmentosa is caused by mutations in the gene encoding the retinitis pigmentosa, the GTPase regulator (\textit{RPGR}) protein, which mainly affects males by the first two decades of life (Figure 3). Female carriers have variable expression, with severe visual disability in some individuals. Multiple preclinical studies have shown photoreceptor rescue with gene therapy.

There are currently several efforts for gene therapy targeting \textit{RPGR}: a U.S. trial sponsored by Applied Genetic Technologies Corp (AGTC) at several locations, a trial by MeiraGTx UK II Ltd in London, and a trial sponsored by Nightstar Therapeutics in Manchester and Oxford with expansion plans in the U.S. in the near future.

**ACHROMATOPSIA**

Achromatopsia is a condition caused by a defect in the cone photoreceptors resulting in poor central vision and abnormal color vision with significant light sensitivity. The affected patients are considered “day-blind” and prefer conditions with dim light.

There are multiple active clinical trials for two genes causing achromatopsia, \textit{CNGA3} and \textit{CNGB3},
which encode the alpha and beta subunits, respectively, for the cone photoreceptor cGMP-gated cation channel.

AGTC sponsors two separate trials, one for each gene at several locations in the U.S., Europe, and Israel.

RETINITIS PIGMENTOSA: MERTK

The Mer proto-oncogene tyrosine kinase gene, or MERTK, encodes a protein located in the retinal pigment epithelium (RPE) involved in the renewal of shed photoreceptor outer segments. Mutations in MERTK lead to accumulation of outer segment debris. In humans, MERTK mutations cause autosomal recessive retinitis pigmentosa and early onset retinal dystrophy. Clinically, these patients have night blindness within the first decade of life, discrete autofluorescent dots, and subretinal debris. In rats, subretinal administration of an AAV vector expressing the MERTK gene resulted in significantly improved electroretinogram (ERG) responses.\textsuperscript{20} Data from rat and Macaque monkey studies support the ocular and systemic safety of the treatment and the viral vector itself. In a phase 1 clinical trial of six patients treated with MERTK gene therapy, no systemic toxicity was observed at 2-year follow-up. Although efficacy was not a primary endpoint, it was noted that three patients had subjective and objective improvement in vision following treatment.\textsuperscript{21}

USHER SYNDROME TYPE I: MYO7A

There are three main clinical types of Usher syndrome — types I, II and III — which is an autosomal recessive disorder characterized by retinitis pigmentosa and sensorineural hearing loss. Patients with Usher syndrome type I, the most severe clinical subtype, have congenital hearing loss, vestibular dysfunction, and early onset retinal dystrophy.\textsuperscript{22} Usher syndrome genes encode proteins involved in intracellular scaffolding and signaling, and when disrupted have negative effects of both photoreceptors and inner ear cells. The myosin VIIa motor protein, MYO7A, is localized to the RPE and the photoreceptor cilia. Dysfunction of MYO7A has been associated with impaired rhodopsin transport and accumulation of rhodopsin in photoreceptor outer segments. MYO7A is the most common cause for Usher syndrome type I.\textsuperscript{9} In deficient mice, subretinal admin-
istration of an equine infectious anemia virus (EIAV) vector expressing the MYO7A gene led to restoration of the deficient protein. Safety studies in macaque monkeys demonstrated safety and tolerability, facilitating initiation of the currently active dose-escalation treatment trial sponsored by Sanofi.23

X-LINKED RETINOSCHISIS

X-linked juvenile retinoschisis (XLRS), characterized by splitting of the retinal layers within the central macula and the peripheral retina, demonstrates abnormal transmission across the photoreceptor-bipolar cell synapse on ERG testing. It is not surprising that mutations in the retinoschisin 1 gene (RS1), contributing to maintaining synaptic structure, are responsible for the development of this retinal disease.24 After the identification of the gene, there have been a number of treatment trials in animal models. In mice, a single intravitreal injection with an AAV vector expressing the RS1 gene resulted in improved ERG responses and decreased cystic cavities on optical coherence tomography.25 Additional gene therapy studies in mice and rabbits further demonstrated safety and tolerability, as well as functional and anatomical improvement.24-28 There are two current separate phase 1 / 2 trials sponsored by the National Eye Institute and AGTC. Both trials employ a strategy of intravitreal treatment to reduce the risk of retinal detachment, with differences in the design of the AAV vector. The animal studies showed a limited immunogenic response with the intravitreal administration, which may be a concern with intravitreal treatment in humans.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Gene</th>
<th>Phase</th>
<th>ClinicalTrials.gov ID(s)</th>
<th>Sponsor(s)</th>
<th>Locations</th>
</tr>
</thead>
</table>
| LCA and retinitis pigmentosa     | RPE65  | 3     | NCT00999609              | Spark, University of Oxford, Nightstar, STZ eyet|xupenn, MEEI, Oxford, BPEI, UTü
| Choroideremia                    | CHM    | 1 / 2 | NCT02341807, NCT02407678, NCT02553135, NCT02671539 | Spark, University of Oxford, Nightstar, STZ eyet| UPenn, MEEI, Oxford, BPEI, UTü
| X-linked retinitis pigmentosa    | RPGR   | 1 / 2 | NCT0316560, NCT03116113, NCT03252847              | AGTC, Nightstar, MeiraGTx                  | Duke, OHSU, UPenn-Scheie, RFSW, Man, UOxford, UCL |
| Stargardt disease                | ABCA4  | 1 / 2a| NCT01736592, NCT01367444 | Sanofi                                          | OHSU, Paris, BPEI, UH, Baylor  |
| Usher syndrome type I            | MYO7A  | 1 / 2a| NCT01505062              | Sanofi                                          | OHSU, Paris                    |
| Achromatopsia                    | CNGA2  | 1 / 2 | NCT02935517, NCT02610582 | AGTC, STZ eyetial                              | VRA, BPEI, MEEI, OHSU, UPenn, Isra, UTü |
| Achromatopsia                    | CNGB3  | 1 / 2 | NCT02599922, NCT03001310 | AGTC and NEI, MeiraGTx                      | VRA, BPEI, OHSU, Chicago, MCW, London |
| X-linked retinoschisis           | RS1    | 1 / 2 | NCT02317887, NCT02416622 | NEI, AGTC                                      | NEI, UCFS, BPEI, JHap, Duke, OHSU, Baylor |
| Retinitis pigmentosa             | MERTK  | 1     | NCT01482195              | King Khaled Eye Specialist Hospital             | Riyadh                         |
| Retinitis pigmentosa             | ChR2   | 1 / 2a| NCT02556736              | Allergan                                        | RFSW                           |
| LCA                             | CEP290 | 1 / 2 | NCT03140969              | ProQR                                           | UH, UPenn-Scheie, Ghent        |

The most advanced completed or active gene therapy treatment trials for retinal dystrophies in the online database clinicaltrials.gov.

LCA = Leber congenital amaurosis; CHOP = Children’s Hospital of Philadelphia; UH = University of Iowa; UPenn = University of Pennsylvania; MEEI = Massachusetts Eye and Ear Infirmary; Oxford = Oxford Eye Hospital; BPEI = Bascom Palmer Eye Institute; UTü = University Hospital Tübingen; Duke = Duke Eye Center; OHSU = Oregon Health and Sciences University; RFSW = Retina Foundation Southwest; Man = Manchester Royal Eye Hospital; UCL = University College London; Baylor = Baylor College of Medicine; VRA = Vitreoretinal Associates Gainesville; Israel = Hadassah-Hebrew Medical Center; AGTC = Applied Genetic Technologies Corp; NEI = National Eye Institute; Chicago = The Chicago Lighthouse; MCW = Medical College of Wisconsin; London = Moorfields Eye Hospital; UCSF = University of California, San Francisco; Ghent = Ghent University Hospital
**STARGARDT DISEASE: ABCA4**

Stargardt disease, an autosomal recessive macular dystrophy, is characterized by lipofuscin accumulation in the RPE, mainly involving the central macula. Mutations in the ABCA4 gene result in accumulation of all-trans-retinal and the toxic byproduct A2E. In ABCA4 gene knock-out mice, the EIAV virus was shown to be an effective vector for the subretinal delivery of the human ABCA4 gene and treated mice had significantly reduced levels of A2E accumulation in the RPE. In patients with Stargardt disease, a Sanofi-sponsored study is in progress to assess the safety and tolerability of ascending doses of subretinal delivery of a lentivirus vector expressing the ABCA4 gene.

**LCA: CEP290**

LCA, caused by the CEP290 gene, which encodes the Centrosomal protein of 290 kDa, is most frequently caused by a specific mutation, c.2991+1655A>G, which alters the gene splicing. Treatment with RNA molecules in the form AONs can significantly improve the normal gene expression in animal models. Patients with LCA affected by this specific mutation in the CEP290 gene are treated every 3 months with intravitreal injections of AONs in a current treatment trial sponsored by ProQR Therapeutics.

**OPTOGENETICS**

Optogenetics is a gene-independent strategy of restoring vision by converting inner retinal neurons to photosensitive cells. Many IRDs have extensive loss of the photoreceptors with remaining bipolar and retinal ganglion cells. Microbial rhodopsins can successfully restore visual function in animal models. In a phase 1/2 trial sponsored by Retrosense Therapeutics, acquired by Allergan in 2017, the channelrhodopsin 2 (ChR2) gene was transduced into the inner retinal neurons in patients with advanced retinitis pigmentosa.

**CONCLUSIONS**

With the recent approval of the first gene therapy treatment for retinal dystrophy caused by the RPE65 gene and emerging gene therapy trials for multiple other disorders, the retina specialist will soon have multiple treatment options for the patients with inherited retinal dystrophies. We have now reached a stage when routine management of IRDs should not be limited to routine exams, but instead patients should have genetic testing for potential gene therapy or possible participation in the growing number of active retinal gene therapy trials.

**REFERENCES**


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