As a small, self-contained, accessible, and relatively immune-privileged organ with a wide range of well-characterized disorders, the eye is an ideal target for gene therapy. This article takes a look at the mechanics of gene therapy, specifically ocular gene therapy, and how gene therapy strategies are being used in clinical trials for inherited retinal diseases (IRDs) and, potentially, for acquired retinal diseases in the near future.

The three general components of ocular gene therapy are (1) the genetic material, which consists of the codon-optimized therapeutic transgene along with promoters, enhancers, and inverted repeats that allow tissue-specific expression of the transgene product; (2) the delivery vehicle to introduce the genetic material; and (3) the route of administration, either via intravitreal or subretinal injection, to target a variety of retinal and choroidal disorders.

The viral vectors (eg, adenovirus, adeno-associated virus [AAV], and lentivirus) that serve as the principal vehicles for the delivery of genetic materials to the eye are modified to remove the pathogenic viral machinery but maintain the components that allow entry into the cell and transit to the nucleus, where the payload (transgene) is transcribed into the desired protein. Viral vectors can be engineered to target specific cell types through modification of the viral capsid (eg, AAV2, AAV8, AAV9, AAV7m8) or via tissue-specific transgene promoters (eg, rhodopsin promoter for targeting rods within the retina).

**GENE THERAPY THREE WAYS**

Gene therapy can be used in a variety of ways, depending on the desired target disease and its underlying cause.

### Gene Augmentation

*Gene augmentation*, in which a functioning copy of an abnormal gene is delivered to the cell, is used in IRDs involving loss-of-function mutations, such as *RPE65*-associated Leber congenital amaurosis (LCA), or to deliver a protein not typically found or made by the target cell. A notable example of the gene augmentation strategy is voretigene neparvovec-ryzl (Luxturna, Spark Therapeutics), the first gene therapy to receive US FDA approval and thereby complete the journey from bench to bedside and enter the realm of clinical reality.

### Gene Inactivation

*Gene inactivation*, by contrast, involves blocking the expression of harmful genes. This approach may be most

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**AT A GLANCE**

- Approaches to gene therapy include gene augmentation, gene inactivation, and gene editing.
- Monogenetic orphan IRDs are good initial targets for gene therapy because of the clarity of the targets and the regulatory incentives for product development.
- Targeted gene therapy has the potential not only to treat IRDs, but also to transform our approach to much more prevalent conditions, such as wet AMD.
appropriate for gain-of-function IRDs such as rhodopsin-linked autosomal dominant retinitis pigmentosa (RP). For gene inactivation to restore normal cellular proteins, it may have to be coupled with gene augmentation to replace the missing gene function.

**Gene Editing**

In gene editing, target cellular DNA is modified to correct specific mutations. Gene editing may be most appropriate for treating IRDs that involve gain-of-function or dominant negative mutations; a specific DNA sequence could be edited out of the target cell and replaced with the sequence of a functioning protein. The clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated-9 (Cas9) system is the current prime example of a gene editing system. The CRISPR/Cas9 system can efficiency and specifically change genes within a variety of cell types. Off-target effects and unwanted mutations elsewhere in the genome of target cells are two important considerations with all gene editing therapeutics.

**GENE THERAPY FOR IRDs**

Several dozen ocular gene therapy trials have been completed, are ongoing, or are in the planning stages. Most of the clinical activity in ocular gene therapy has centered around monogenetic IRDs. The reasons for this include the precise identification of the target gene and the clear and relatively straightforward path to FDA approval. That is, most IRDs are considered by the FDA to be orphan diseases, defined as diseases that affected fewer than 200,000 people in the United States, and therefore most treatments can gain breakthrough therapy designation. The incentives for orphan product and breakthrough therapy designations include a less cumber-some regulatory timeline and potential 7-year exclusivity once the product is approved.

A look at the genetic targets and the mechanisms being used in some of these trials follows. See also the article by Benjamin Bakall, MD, on page 40 of this issue for additional details on some of these conditions and the therapies being studied for their treatment.

**RPE65-MEDIATED DISEASE**

The RPE65 gene, expressed in retinal pigment epithelial (RPE) cells, encodes a carotenoid oxygenase enzyme that converts 11-trans-retinyl esters to 11-cis-retinol, which is then used in visual pigment regeneration in photoreceptors. Mutations in RPE65 have been associated with LCA type 2 (LCA2) and RP, specifically RP20.

Voretigene was approved by the FDA in December for the treatment of patients with biallelic mutations in the RPE65 gene. The first patient outside a clinical trial was treated in March. Patients have now been treated in six centers in the United States, according to the drug’s manufacturer. Voretigene is an AAV-based gene therapy that is delivered into the subretinal space after pars plana vitrectomy to patients with genetically confirmed IRD secondary to mutations in both copies of the RPE65 gene. Subretinal injection of voretigene delivers a normal copy of the RPE65 gene to the RPE cells, which then produce the functioning RPE65 protein and restore the visual cycle.

MeiraGTx is also developing an AAV-RPE65 gene therapy for patients with biallelic mutations in the RPE65 gene. This approach uses a codon-optimized RPE65 gene that is driven by a novel synthetic RPE cell-specific promoter that is said to be 100 to 1,000 times more potent than the first-generation therapy. A phase 1/2 clinical study has completed dosing of nine adults in three escalating dose cohorts and six pediatric patients in a pediatric extension arm. Results are forthcoming.

**CHOROIDEREMIA**

Choroideremia is an X-linked recessive IRD resulting from a loss-of-function mutation in the CHM gene that encodes Rab escort protein 1 (REP1). Lack of functional REP1 leads to cell death and progressive loss of choroid, RPE, and photoreceptors, which results in blindness. Two gene therapies are being assessed in clinical trials.

**SPK-7001**

A prospective 2-year phase 1/2 open-label clinical trial of SPK-7001 (Spark Therapeutics) is ongoing. In an interim analysis, SPK-7001 resulted in no product-related serious adverse events. Four of 10 later-stage participants showed non–statistically significant indications of efficacy on one or more endpoints. This may have been due to the late stage of the disease in this cohort. An additional cohort of five patients with earlier
stage disease has completed enrollment, and further safety and efficacy analysis is expected to be reported in late 2018.

**NSR-REP1**

Across four open-label phase 1/2 clinical trials including 32 participants, more than 90% of patients treated with NSR-REP1 (Nightstar Therapeutics) maintained or improved visual acuity over a 1-year period. Based on these encouraging results, the company announced the initiation of a phase 3 trial to study the safety and efficacy of NSR-REP1 in 140 patients with choroideremia. The 12-month primary endpoint will be the proportion of patients with a 15-letter improvement from baseline visual acuity.

**X-LINKED RETINITIS PIGMENTOSA**

Mutations in the gene for the RP GTPase regulator protein (RPGR) have been associated with approximately 70% of X-linked RP (XLRP). Because RPGR is involved in the transport of proteins responsible for maintenance of photoreceptor health, loss of RPGR function results in progressive death of rods and cones. Men with RPGR mutations typically develop vision loss in the first 2 decades of life, starting with night and peripheral vision difficulties during childhood and progressing to central vision loss in their 20s and 30s. Most women who are carriers are asymptomatic, but some may develop vision loss similar to that seen in men. Several gene therapy trials in XLRP are in progress.

**NSR-RPGR**

Nightstar Therapeutics has initiated a phase 1/2 clinical trial for the treatment of XLRP using an AAV vector with a codon-optimized RPGR gene (NSR-RPGR) that results in higher protein expression compared with that of a wild-type RPGR coding sequence. NSR-RPGR is designed to produce the RPGR open reading frame 15 (RPGR-ORF15) protein, the configuration of RPGR expressed in the retina.

**AAV-RPGR**

MeiraGTx is conducting a phase 1/2 clinical trial of its product candidate AAV-RPGR in adult and pediatric patients with XLRP. In a dose escalation phase, up to 18 adults will receive one of three escalating doses. Once a suitable dose is determined in adults, the trial will expand to treat up to 12 children with RPGR mutations.

In July, Applied Genetic Technologies Corporation (AGTC), in collaboration with Biogen, enrolled the first patient in a phase 1/2 open-label, dose escalation study of subretinal administration of an AAV-based gene therapy in patients with XLRP caused by RPGR gene mutations. Up to 15 patients will be enrolled.

**X-LINKED RETINOSCHISIS**

X-linked retinoschisis (XLRS) is characterized by an abnormal splitting of the neurosensory retina, often involving the central macula, resulting in decreased visual acuity from an early age. XLRS is caused by an abnormality in the RS1 gene, which encodes a protein, retinoschisin, that is secreted by the outer retina and is thought to be involved in cell-cell adhesions and retinal intracellular matrix development. Deficiency in retinoschisin results in retinal cavities, retinal synaptic dysfunction, reduced visual acuity, and predisposition to retinal detachments.

**AAV8-RS1**

The National Institutes of Health’s National Eye Institute recently reported initial findings from a phase 1/2 study of intravitreal AAV8-RS1 in patients with XLRS. In this single-center, dose-escalating, prospective, open-label clinical trial, the intravitreal vector was administered to nine patients with pathogenic RS1 gene mutations. AAV8-RS1 was generally well tolerated, with dose-related ocular inflammation and dose-related increases in systemic AAV8 antibodies noted. The retinal cavities closed transiently in one treated patient in the higher dose group. Additional doses and use with immunosuppressive regimens are being explored.

**rAAV2tYF-CB-hRS1**

AGTC, in collaboration with Biogen, has completed enrollment in a phase 1/2 clinical trial of rAAV2tYF-CB-hRS1 in patients with XLRP caused by mutations in the RS1 gene. Approximately 27 patients were enrolled sequentially in four dose-escalating groups, with the fourth group receiving the maximum tolerated dose as determined by the first three groups. A group of pediatric patients was also enrolled, receiving the middle dose. In addition to the primary endpoint of safety, the trial will evaluate visual function, retinal structure, and quality-of-life measures after gene therapy administration. Top-line data from this study are anticipated in late 2018, with final analysis at 12 months.

**ACHROMATOPSIA**

Congenital achromatopsia, or rod monochromacy, is an autosomal-recessive disorder characterized by loss of cone function. Abnormalities in five genes, all encoding proteins required for critical steps of the phototransduction pathway in cones, have been linked to achromatopsia. Mutations in the gene encoding cyclic nucleotide-gated channel beta 3 (CNGB3/ACHM3) is thought to be responsible for more than 50% of achromatopsia, and mutations in cyclic nucleotide-gated channel alpha 3 (CNAG3/ACHM2) is responsible for close to 25%. The CNGB3 and CNAG3 genes encode the two subunits of the cyclic nucleotide-gated (CNG) channel expressed in cone outer segments. Three CNGB3 and one CNAG3 subunits combine to form the cone CNG channel that mediates the transduction of light-triggered changes necessary for the depolarization of cone photoreceptor cells.

**AAV-CNGB3**

MeiraGTx is conducting a dose-escalating phase 1/2 open-label clinical trial of subretinally administered AAV-CNGB3 in adult and pediatric patients with CNGB3-associated achromatopsia. Up to 18 adult patients will receive one of three doses of
AAV-CNGB3. Once an acceptable dose is established, up to nine pediatric patients will be treated.

AGTC is conducting two separate phase 1/2 clinical trials to evaluate the safety and efficacy of AAV-delivered gene therapy for the two most common forms of achromatopsia: those caused by a mutation in either the CNGB3 or CNGA3 genes.11,12

Both studies are enrolling patients.

OTHER IRDs
In addition to the gene therapy approaches described above, which are at or beyond the phase 1/2 trial stage, there are more than a dozen gene therapy product candidates for a variety of disorders in late preclinical or early phase 1 stage development. Follow Retina Today for more on these product candidates in future issues.

GENE THERAPY FOR ACQUIRED DISORDERS
The approval of voretigene has served as a proof of concept for ocular gene therapy as a viable therapeutic option for the treatment of single-gene IRDs, and clearly there are many more such approaches in the pipeline. Perhaps more exciting, however, is the potential for application of gene therapy techniques to more multifactorial, often noninherited, disorders, such as age-related macular degeneration (AMD)13-15 and diabetic retinopathy. Such therapies would represent a true paradigm shift for millions of patients, rather than the thousands or tens of thousands affected by IRDs.

The overarching concept for gene therapy in these types of diseases would be to introduce a transgene that is not otherwise found in the target cell (eg, an anti-VEGF molecule for the treatment of wet AMD), rather than to fix an inherited genetic abnormality. The eye could then become a biofactory that produces the transgene indefinitely, obviating the need for repeated intravitreal anti-VEGF injections, for example, in the case of patients with wet AMD.

Several dose-escalating phase 1/2 trials of therapies for wet AMD failed to show sufficient efficacy to move further in development. Nevertheless, several companies are evaluating gene therapy product candidates for wet AMD and for dry AMD. These investigations are in phase 1 or preclinical stages. We can look forward to further development in this area of inquiry.

MORE TO COME
No doubt, 2018 will be remembered as the year when gene therapy entered our therapeutic armamentarium. As targeted gene therapy treatment options for IRDs continue to expand, mostly in the form of an ever-growing number of clinical trials, genetic testing for more precise molecular diagnosis of patients with retinal degenerations will become increasingly important. (See page 55, where Christine Kay, MD, examines the role of genetic testing as a new standard of care for IRDs.)

Although our experience with monogenetic IRDs has provided proof of the value of gene therapy, its true promise is its potential to transform our approach to treating much more prevalent conditions, such as wet AMD. Continued success for ocular gene therapy will undoubtedly depend on advances in capsid selection (eg, AAV8, AAV9, AAV7m8), gene cassette optimization (including choice of promoters and enhancers), formulation, manufacturing, and appropriate route of delivery (intravitreal vs subretinal).