Precision Therapy for Inherited Retinal Disease

At the Forefront of Genomic Medicine

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KEYWORDS

- Adeno-associated virus (AAV)
- Clustered regularly interspaced short palindromic repeats (CRISPR) • Gene transfer
- Inherited retinal disease • Next-generation sequencing (NGS) • Retinal degeneration
- Sanger sequencing • Subretinal gene therapy

KEY POINTS

- Next-generation sequencing enables rapid and inexpensive whole-genome and exome sequencing. The Sanger method remains vital for variant confirmation and deep intronic sequencing.
- The retina is ideal for gene therapy because it is accessible, is relatively immune privileged, requires only small volumes of medicine, and has measurable anatomic and functional endpoints.
- Food and Drug Administration approval of voretigene neparvovec-rzyl (Luxturna) represents a landmark in the field, with this gene therapy now being delivered at several centers in the United States.
- A rapidly expanding number of gene therapy trials are under way for patients with achromatopsia (*CNGA3* and *CNGB3*), choroideremia (*CHM*), Stargardt disease (*ABCA4*), retinitis pigmentosa (*RPGR*, *MERTK*, and *PDE6B*), Usher syndrome (*MYO7A*), and X-linked retinoschisis (*RS1*).
- Novel gene targeting techniques, such as clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9, antisense oligonucleotides, and optogenetics also are being employed.

INTRODUCTION

Inherited retinal diseases (IRDs) are a genotypically and phenotypically heterogenous group of disorders affecting the retina. The neurosensory retina is a thin, multilayered structure lining the inner wall of the eye, composed of an intricate network of cells,

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including photoreceptors, bipolar cells, retinal ganglion cells, and various interneuron subtypes responsible for transducing light stimuli into electrical signals. The peripheral retina is rich in rod photoreceptors, which are important for peripheral vision and low-light vision. In contrast, the macula, whose central 1.5-mm diameter area is known as the fovea, is rich in cone photoreceptors, which are important for high spatial acuity and color vision processing.[1]

Light travels through the full thickness of the retina to the photoreceptor cells, whose outer segments are the site of visual transduction. Essential to the function of these photoreceptors is a monolayer of support cells adjacent to the outer segments, known as the retinal pigment epithelium (RPE).[1] The pathophysiology of many IRDs is believed to arise from dysfunction or loss of the RPE, rod photoreceptors, and/or cone photoreceptors.

IRDs represent a significant source of visual impairment in all age ranges and are thought to affect 200,000 individuals in the United States.[2] These disorders typically affect the retina bilaterally and symmetrically, and syndromic forms may have associated systemic findings, such as sensorineural deafness, nephropathy, neurologic impairment, and other findings. The clinical diagnosis typically is made by clinical examination with adjunct diagnostic testing, which includes optical coherence tomography (OCT), fundus autofluorescence, electroretinography (ERG), and visual field testing. Given the heterogenous genetic nature of IRDs and the extreme specificity of the current treatment modalities, however, genetic testing has become an essential element of diagnosis.[3]

MOLECULAR DIAGNOSTIC TESTING FOR RETINAL DYSTROPHIES

The development of next-generation sequencing (NGS) has changed the field of molecular diagnostics by enabling rapid, high-throughput, whole-genome and whole-exome sequencing at significantly lower costs. Using this technology, targeted panels of known retinal dystrophy genes and candidate genes can be performed at many institutions and commercial laboratories.[4] This technology has greatly improved the diagnostic yield and accuracy of testing, but traditional Sanger sequencing continues to play a role in confirming mutations and for sequencing of deep intronic mutations (eg, *CEP290*) and guanine-cytosine nucleotide-rich areas (eg, *RPGR*), which are poorly read by NGS. The field has come a long way since the first IRD gene was reported in 1984 in relation to X-linked retinitis pigmentosa (RP).[5] Since then, there has been a steady rise in the number of genes identified ,with 271 genes linked to IRDs as of 2019 (**Fig. 1**).[6]

NGS not only has facilitated the identification of causative retinal disease genes but also now serves to identify candidates for gene therapy. This is a reflection of significant genetic heterogeneity among patients with IRDs, the difficulty of phenotype-genotype correlation for many conditions (**Fig. 2**), and the need for diagnostic certainty prior to proceeding with gene therapy. Given the risks of bilateral gene therapy surgery, the establishment of a sound genetic diagnosis is crucial and must be supported by segregation analysis, up-to-date variant databases and prediction algorithms, and in some cases in vitro verification of mutational pathogenicity.[7]

GENE THERAPY FOR RETINAL DYSTROPHIES

Over the past 2 decades, a dramatic explosion in understanding of retinal dystrophies, vitreoretinal surgical techniques, and viral vectors[8–10] has created fertile ground for addressing these diseases with gene replacement and other allele-targeting strategies. In most cases, the goal is gene replacement, using a normal copy of the diseased

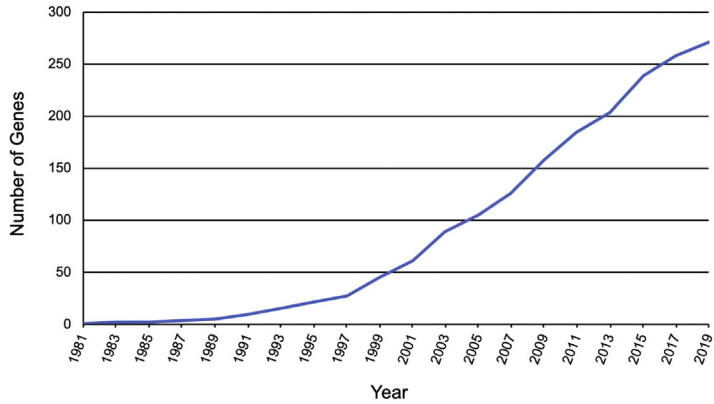

Fig. 1. Number of retinal disease genes identified over the past 38 years. Since the first IRD gene was reported in 1984, there has been a dramatic rise in the number of IRD-related genes identified. As of 2019, 271 genes have been discovered.

gene. Other strategies involve targeting diseased alleles with mutation-specific or exon-specific antisense oligonucleotides.

Viral Vectors for the Retina

To date, several viral vectors, including lentivirus, adenovirus, and adeno-associated virus (AAV), have been explored for use in gene therapy (**Table 1**). Advantages of the lentivirus vector include its large complementary DNA (cDNA) packing capacity (8–10 kilobases [kb]) and its ability to efficiently transduce RPE cells. Disadvantages include its less effective ability to target differentiated photoreceptors and its risk for insertional mutagenesis as it integrates into the target cell's genome.[11] AAV has overwhelmingly become the viral vector of choice for ocular gene delivery, despite its smaller packaging capacity (4.8 kb). This recombinant, nonenveloped, single-stranded, DNA parvovirus has a favorable immunogenicity and toxicity profile, and, by modifying the viral capsid and promoter region, it can be targeted to express protein in specific cell types of the retina.[12] Use of adenovirus has been limited due to its high immunogenicity.

The Retina as a Target for Gene Therapy

The retina offers several advantages for gene therapy.[12] First, the relative immune-privileged status of the subretinal space minimizes host response to viral vector.[13] Second, intravitreal and especially subretinal delivery approaches require a relatively small volume of vector in contrast to systemic delivery.[14] Third, the anatomic accessibility of the retina allows for a direct view of the target tissue during vector delivery and enables noninvasive, multimodal, functional, and anatomic monitoring of the delivery site in an outpatient setting. Finally, given the relatively symmetric nature of disease progression in IRDs, the contralateral eye can serve as a control when assessing safety and efficacy.[15]

Surgical Techniques for Vector Delivery

Intravitreal and subretinal injections are the main techniques for gene therapy delivery for retinal dystrophies. The intravitreal approach is the most straightforward because it requires only topical anesthesia and is routinely performed in the outpatient setting for

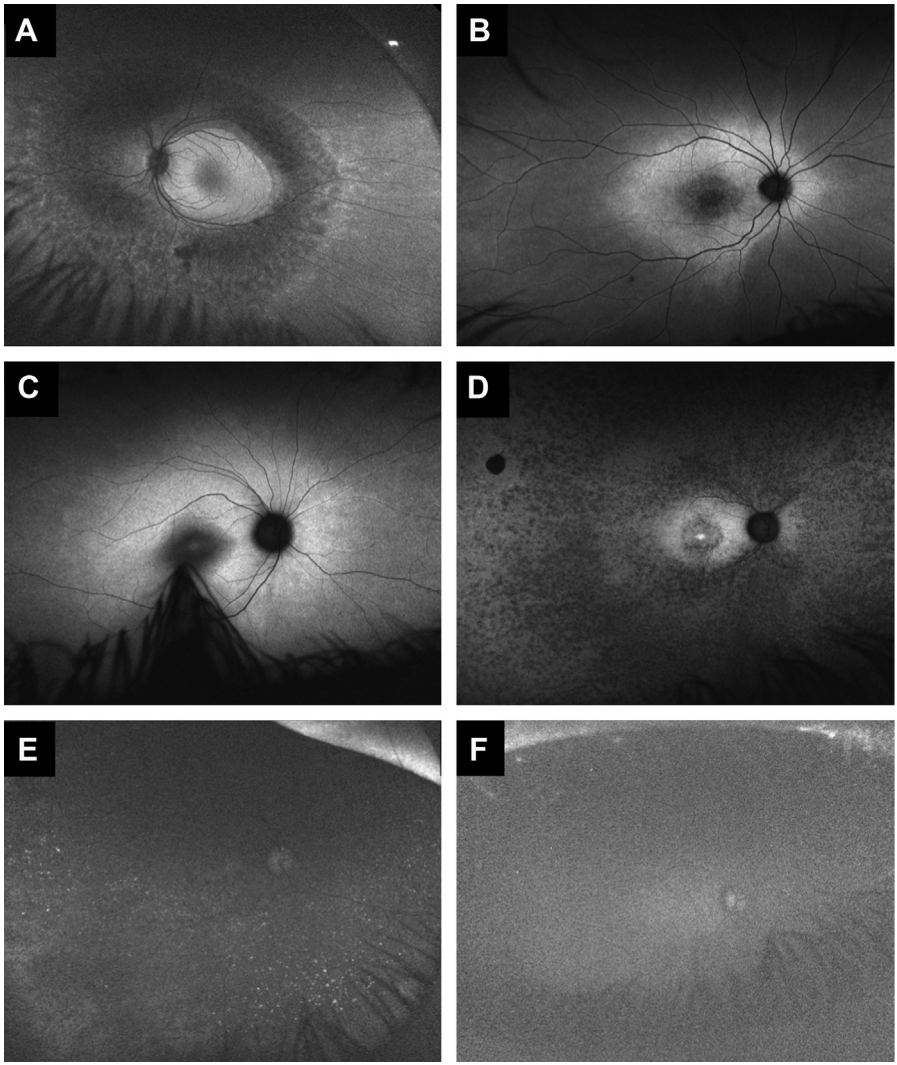

Fig. 2. A sampling of autofluorescence photographs demonstrating the diversity of retinal phenotypes and genotypes. Note that only 1 eye of each patient was included, but that the fellow eye had highly symmetric findings. (*A*) The left eye of a 10-year-old boy with enhanced S-cone syndrome (*NR2E3* c.119-2A > C and c.1142 T > G) features hypoautofluorescence around the vascular arcades. (*B*) In contrast, the right eye of a 6-year-old girl with Stargardt disease (*ABCA4* c.6146delA and c.2424 C > G) demonstrates central hypoautofluorescence with surrounding hyperautofluorescence. (*C*) A similar autofluorescence pattern is seen in an 8-year-old boy with Bardet-Biedl syndrome type 6 (BBS6 c.110 A > G and c.415 C > T). (*D*) In contrast, a 14-year-old girl with Bardet-Biedl syndrome type 1 has marked peripheral hypoautofluorescence and central hyperautofluoresence (*BBS1* c.1169 T > G and c.1181-9C > G). The patients in (*C*) and (*D*) both had associated systemic findings, including polydactyly, cognitive impairment, and truncal obesity. Autofluorescence of LCA associated with (*E*) the *CRB1* gene (homozygous c.2501 G > A) and (*F*) the *RPE65* gene (homozygous c.917 C > A) .

Table 1
Comparison of viral vectors for retinal gene therapy

Vector Type	Genome	Packaging Capacity	Integrates into Target Cell Genome	Infects Dividing and Nondividing Cells	Retinal Cell Targets	Immune Response	Relative Viral Titers	Relative Transduction Efficiency
AAV	4.8 kb (ssDNA)	4.7 kb	No	Yes	RPE, Müller, PRs, GCs	Very low	Moderate	Moderate
Lentivirus	9 kb (ssRNA)	8.0–10.0 kb	Yes	Yes	RPEs > PRs	Low	Moderate	Moderate
Adenovirus	36 kb (dsDNA)	7.5 kb	No	Yes	RPE, Muller	High	High	High

Abbreviations: ds, double-stranded; GCs, ganglion cells; Müller, Müller cells; PRs, photoreceptors; ss, single-stranded.

other indications. There are concerns, however, with accessibility of the vector to photoreceptors and RPE as well as issues with inflammation. Therefore, most trials currently utilize subretinal delivery via pars plana vitrectomy. This approach is familiar to most surgeons but poses a small risk of cataract and retinal detachment. Surgical techniques for subretinal delivery have been optimized and refined.[16] Instrumentation, such as the MicroDose Injection Kit (MedOne Surgical, Sarasota, Florida), allows for pedal-controlled delivery of the viral vector to the subretinal space rather than reliance on manual delivery via an assistant. Intraoperative OCT has become a valuable tool by permitting real-time bleb visualization to ensure subretinal location and monitoring of the fovea to minimize stretch (**Fig. 3**).[17] Novel approaches include ab externo subretinal injection via the suprachoroidal space, which is advantageous in that no vitrectomy or retinotomy are required.[18]

Voretigene Neparvovec-rzyl: Paving the Way

The landmark results of the gene therapy trial for *RPE65*-mediated Leber congenital amaurosis (LCA) led to the US Food and Drug Administration (FDA) approval of voretigene neparvovec-rzyl (Luxturna; Spark Therapeutics, Philadelphia, Pennsylvania) in December 2017. Voretigene neparvovec-rzyl represents the first FDA-approved gene replacement for a hereditary condition and resulted from almost 3 decades of research spearheaded by Jean Bennett, Albert Maguire, Michael Redmond, and many others. Sixteen years after demonstrating efficacy in a canine model,[19] Russell and colleagues[20] published the results of their phase III clinical trial (NCT00999609) in 2017, which showed that treated participants demonstrated significantly improved light sensitivity, visual fields, and ability to navigate in low-light conditions. These statistically significant changes were apparent at 30 days and persisted at 1 year and 4 years after treatment.[21]

Currently, voretigene neparvovec-rzyl gene therapy is performed by selected vitreoretinal surgeons at nine institutions across the United States. The treatment is

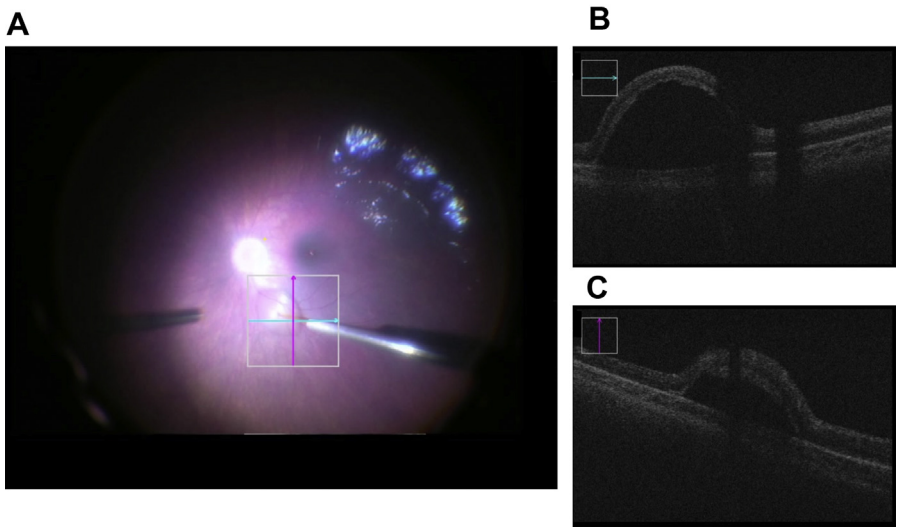

Fig. 3. Use of intraoperative OCT during voretigene neparvovec-rzyl delivery. (*A*) Surgical view during delivery of voretigene subretinally with a 38-gauge tip cannula. The live 2-line cross-hair OCT images (horizontal, B; vertical, C) are displayed on the surgical microscope, providing confirmation of subretinal delivery.

performed via vitrectomy followed by subretinal injection of 1.5×10^{11} vector genomes of voretigene neparvovec-rzyl, delivered to the subretinal space in a total volume of 0.3 mL. Typically, the injection site is along the superotemporal vascular arcade at least 2 mm away from the fovea. At this time, the medication costs approximately $425,000 per eye in the United States and will be available in Europe shortly.

Current Retinal Gene Augmentation Trials

Gene therapy for *RPE65*-mediated LCA is a reality, but clinical trials for other retinal dystrophies are moving forward at a rapid pace. To date, there are 27 clinical trials under way using precise gene-specific or allele-specific approaches to treat these conditions (**Table 2**).

Achromatopsia (CNGA3 and CNGB3)

Achromatopsia is a cone dystrophy characterized by severe hemeralopia (day blindness), severe color blindness, and reduced visual acuity.[22] There currently are 6 genes implicated in achromatopsia: *CNGA3*, *CNGB3*, *GNAT2*, *PDE6C*, *PDE6H*, and *ATF6*, of which *CNGB3* and *CNGA3* mutations account for 50% and 25% of all cases, respectively.[23,24] Currently, 5 phase I/II clinical trials are under way for gene delivery of *CNGB3* (NCT02599922 and NCT03001310) and *CNGA3* (NCT02935517, NCT03758404, and NCT02610582).

Choroideremia (CHM)

Choroideremia is an X-linked retinal dystrophy characterized by loss of RPE and secondary degeneration of the choriocapillaris and photoreceptors, and manifests with nyctalopia and progressive peripheral field constriction. It primarily affects men and is caused by variants or deletions in the *CHM* gene, which encodes Rab-escort protein 1 (REP1).[25] The 2-year outcomes of the first phase I/II clinical trial for choroideremia (NCT01461213) showed a statistically significant and sustained improvement of visual acuity in treated eyes (median 4.5 letter gain vs 1.5 letter loss; $P = .04$).[26] These outcomes have been confirmed in other trials (NCT02077361,[27] NCT02553135,[28] and NCT02671539[29]), and there are several ongoing or recruiting trials (NCT02341807, NCT02407678, and NCT03507686), including a phase III trial (NCT03496012).

Stargardt Disease (ABCA4)

Autosomal recessive Stargardt disease has a prevalence of 1 in 10,000 and results from mutations in the *ATP-binding cassette, subfamily A, member 4* (*ABCA4*) gene.[30] Vision loss ensues early in life, typically before adolescence owing to atrophy of the macular RPE and photoreceptors. Its pathophysiology includes impaired trafficking and impaired clearance of *N*-retinylidene phosphatidylethanolamine (a retinoid intermediate) from the outer segments of rod and cone photoreceptors, which leads to the pathologic accumulation of lipofuscin in the RPE.[31] Gene therapy offers potential for the treatment of Stargardt, but the size of the *ABCA4* gene is too large (6.8 kb) to be packaged in an AAV vector. Using a lentivirus with a larger cDNA capacity, Sanofi has undertaken a phase I/IIa dose escalation safety study of subretinally injected SAR422459 in 27 subjects (NCT01367444) and followed-up with a larger trial with 46 subjects (NCT01736592). This work is ongoing and results have not yet been published.

Retinitis Pigmentosa (RPGR, MERTK, and PDE6B)

RP is a heterogeneous group of retinal dystrophies characterized by progressive degeneration of rod photoreceptors, followed by secondary degeneration of cone

Table 2
Current clinical trials for inherited retinal diseases

Disease	Gene	Phase	Clinical Trial	Viral Vector/Drug	Sponsor	Mode of Delivery
Achromatopsia	*CNGA3*	I/II	NCT02935517	rAAV2tYF-PR1.7-hCNGA3	Applied Genetic Technologies Corporation	Subretinal
	CNGA3	I/II	NCT03758404	AAV2/8-hG1.7p.coCNGA3	MeiraGTx UK II Limited	Subretinal
	CNGA3	I/II	NCT02610582	rAAV8.hCNGA3	STZ Eyetrial	Subretinal
	CNGB3	I/II	NCT02599922	rAAV2tYF-PR1.7-hCNGB3	Applied Genetic Technologies Corporation	Subretinal
	CNGB3	I/II	NCT03001310	AAV2/8-hCARp.hCNGB3	MeiraGTx UK II Limited	Subretinal
Choroideremia	*CHM*	III	NCT03496012	AAV2.REP1	Biogen/NightstaRx Limited	Subretinal
		II	NCT02407678			
		II	NCT03507686			
		II	NCT02671539			
	CHM	I/II	NCT02341807	AAV2-hCHM	Spark Therapeutics	Subretinal
LCA	*RPE65*	III	NCT00999609	AAV2-hRPE65 v2 (voretigene neparvovec-rzyl)	Spark Therapeutics	Subretinal
		I/II	NCT01208389			
		I	NCT00516477			
	RPE65	I	NCT00481546	rAAV2-CBSB-hRPE65	University of Pennsylvania	Subretinal
	RPE65	I/II	NCT02781480	AAV2/5-OPTIRPE65	MeiraGTx UK II Limited	Subretinal
	GUCY2D	I/II	NCT03920007	AAV-GUCY2D (SAR439483)	Sanofi	Subretinal
	CEP290(c.2991 + 1655A > G)	I/II	NCT03872479	CRISPR/Cas9-IVS26 (AGN-151587)	Allergan	Subretinal
	CEP290(c.2991 + 1655A > G)	II/III	NCT03913143	Antisense oligonucleotide to IVS26 pre-mRNA (QR-110)	ProQR Therapeutics	Intravitreal
		I/II	NCT03140969			
		I/II	NCT03913130			

RP	*RPGR*	II/III	NCT03116113	AAV8-RPGR	Biogen/NightstaRx	Subretinal
	RPGR	I/II	NCT03316560	rAAV2tYF-GRK1-RPGR	Applied Genetic Technologies Corporation	Subretinal
	RPGR	I/II	NCT03252847	AAV2-RPGR	MeiraGTx UK II	Subretinal
	PDE6B	I/II	NCT03328130	AAV2/5-hPDE6B	Horama S.A.	Subretinal
	RLBP1	I/II	NCT03374657	AAV8-RLBP1 (CPK850)	Novartis	Subretinal
	MERTK	I	NCT01482195	rAAV2-VMD2-hMERTK	King Khaled Eye Specialist Hospital	Subretinal
	n/a	I/II	NCT03326336	rAAV2.7m8-CAG-ChrimsonR-tdTomato (GS030-DP)	GenSight Biologics	Intravitreal
	n/a	I/II	NCT02556736	AAV2-channelrhodopsin-2	Allergan	Intravitreal
	RHO(P23H)	I/II	NCT04123626	antisense oligonucleotide to P23H mRNA (QR-1123)	ProQR Therapeutics	Intravitreal
Usher syndrome IB	*MYO7A*	I/II	NCT02065011	EIAV-CMV-MYO7A (UshStat)	Sanofi	Subretinal
Usher syndrome 2A	*USH2A(Exon13)*	I/II	NCT03780257	antisense oligonucleotide to USH2A Exon13 pre-mRNA (QR-421A)	ProQR Therapeutics	Intravitreal
Stargardt disease	*ABCA4*	I/II	NCT01736592	Lentivirus-ABCA4 (SAR422459)	Sanofi	Subretinal
XLRS	*RS1*	I/II	NCT02317887	AAV8-scRS/IRBPhRS	National Eye Institute	Intravitreal
	RS1	I/II	NCT02416622	rAAV2tYF-CB-hRS1	Applied Genetic Technologies Corporation	Intravitreal

photoreceptors and RPE cells. Symptoms typically include nyctalopia, progressive peripheral field loss, and, in late states, decreased central visual acuity due to cone photoreceptor degeneration. More than 200 genes and upwards of 3000 mutations have been implicated in RP.[32] Inheritance patterns vary and include autosomal recessive (50%–60% of cases), autosomal dominant (30%–40%), and X-linked recessive (5%–15%).

Mutations in the *retinitis pigmentosa GTPase regulator* (*RPGR*) gene have been identified in more than 70% of X-linked recessive families.[32] Currently, there are 3 clinical trials for *RPGR*-associated RP using three different viral vectors; these include 2 phase I/II clinical trials (NCT03316560, NCT03252847) and 1 phase II/III study (XIRIUS, Biogen/NightStaRx; NCT03116113), whose phase I/II dose escalation study has thus far shown positive preliminary safety and efficacy data, including improved central retinal sensitivity.[33,34] The groundwork for all 3 clinical trials comes in large part from prior work done on codon optimization of the *RPGR* sequence, and experiments in 2 *RPGR* mouse models (*RPGR*-KO and Rd9) that showed improved ERG responses in treated mice at 4 months and 6 months.[35,36]

Gene replacement therapy for *MER proto-oncogene tyrosine kinase* (*MERTK*)-associated RP is another example. Efficacious results were shown in *MERTK* mutant rats (Royal College of Surgeons) who underwent subretinal delivery of the human *MERTK* construct (AAV2-VMD2-hMERTK),[37] as part of the preclinical work for the phase I clinical trial (NCT01482195). Thus far, the preliminary results have shown that the vector generally is well tolerated, and 50% of subjects (3/6) demonstrated measurable improvements in visual acuity, although the effect was lost by 2 years in 2 of 3 patients.[38]

Another phase I/II clinical trial under way for RP utilizes an AAV2 vector for delivery of the *phosphodiesterase 6-beta subunit* (*PDE6B*) gene (NCT03328130). *PDE6B*-mediated RP represent 4% to 5% of all RP cases.[39] In the canine model, AAV2 delivery of the *PDE6B* gene halted rod degeneration at 3.5 years follow-up.[40]

Usher Syndrome Type I (MYO7A)

Usher syndrome is a form of syndromic RP affecting 1 in 25,000 people and characterized by sensory impairment of the visual and the audiovestibular systems.[41] Usher syndrome is a heterogeneous disease with 3 clinical subtypes and 9 associated genes.[42] Usher syndrome 1B is caused by mutations in *MYO7A*, which encodes myosin VIIA, an important protein for ciliary transport between photoreceptor inner and outer segments. Deafness and photoreceptor dysfunction manifest at birth with ensuing retinal degeneration. A phase I/IIa trial by Oxford Biomedica (Oxford, UK) (NCT01505062) is evaluating *MYO7A* gene therapy (UshStat) for patients with Usher syndrome, Type 1B. The size of the transgene that expresses myosin VIIA is too large for the AAV vector; thus, the trial employs a subretinal injection of the gene product using an equine lentiviral-based vector. Four patients have been enrolled with good safety outcomes but final results are still pending.[43]

X-Linked Retinoschisis (RS1)

X-linked retinoschisis (XLRS) is an inherited retinal dystrophy in boys that is caused by mutations in the *retinoschisin 1* (*RS1*) gene and characterized by schisis of the retinal layers leading to impaired synaptic transmission from photoreceptors to bipolar cells.[44] The impetus for clinical trials in humans stems from work done in the murine model, which showed that intravitreal delivery of human *RS1* gene (AAV8-RS1) led to restoration of the retinoschisin peptide in its typical histologic distribution, resolution of schisis cavities, and improved ERG b-wave responses.[45] Two phase I/II dose escalation clinical trials for XLRS have been performed (NCT02317887 and NCT02416622) using intravitreally delivered vector.

In the trial (NCT02317887) led by the National Eye Institute, the vector (AAV8-scRS/IRBPhRS) required topical and/or systemic steroids in all 9 patients due to vector-related inflammation. All visual outcome parameters, including visual acuity, returned to baseline by 18 months.[46] The other clinical trial (NCT02416622), led by Applied Genetic Technologies Corporation (Alachua, Florida), showed similar disappointing results and the trial was terminated. Future work is aimed at optimizing the delivery, dose, and immunosuppressive regimen.[47]

NOVEL GENE THERAPY APPROACHES

In addition to the many gene replacement trials, novel approaches are being employed to achieve mutation-specific or allele-specific targeting. Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 is an RNA-guided nuclease technique, which represents an exciting potential avenue for diseases not amenable to traditional gene replacement, such as autosomal dominant conditions.[48] CRISPR-Cas9–mediated DNA breaks can be repaired either through homology-directed repair, allowing for knock-in of DNA sequences, or through nonhomologous end joining, which often results in insertions or deletions of random nucleotides at the site (indels). Thus, it may be possible to not only destroy pathogenic alleles but also to perform CRISPR-mediated gene correction in situ or on patient-derived induced pluripotent stem cells in order to generate gene-corrected differentiated cells (eg, RPE or photoreceptors) for autologous cellular transplantion.[3,49,50]

Editas Medicine (Cambridge, MA), in partnership with Allergan (Dublin, Ireland), announced in July 2019 the initiation of the Brilliance phase I/II clinical trial of subretinal AGN-151587 (NCT03872479) for the treatment of LCA caused by the deep intronic *c.2991 + 1655A > G* (*IVS26*) mutation in the *CEP290* gene.[51] This represents the first in vivo trial of a CRISPR-based therapy to achieve targeted deletion of a cryptic splice site caused by the IVS26 mutation and restore expression of CEP290 protein.[52] Concerns with this treatment strategy include persistence of Cas9 activity and off-target effects at other genomic loci.[52,53]

Other novel therapies for the treatment of IRDs include the use of antisense oligonucleotides to target aberrant pre–messenger RNAs (pre-mRNAs) implicated in IRDs, thereby modulating mRNA splicing and/or stability in an allele-specific fashion.[54] Rather than use CRISPR methods to delete the *CEP290* cryptic splice site, ProQR Therapeutics (Leiden, Netherlands) is assessing the safety and efficacy of intravitreally injected antisense oligonucleotide (QR-110) to suppress the IVS26-associated cryptic splice site (NCT03913143, NCT03140969, and NCT03913130).[55]

Other antisense-based clinical trials illustrate the degree to which precision medicine is being utilized for the treatment of IRDs. An intravitreal antisense oligonucleotide that is mutation-specific is being employed against mutant *RHO(P23H)* mRNA in patients with dominant *RHO*-associated RP (NCT04123626; QR-1123). Another example is the use of antisense to induce exon-skipping for Usher Syndrome, Type 2A patients with pathogenic variants in exon 13 (NCT03780257; QR-421A).

THE FUTURE OF THE FIELD

Gene therapy is undergoing rapid evolution and expansion as the number of trials dramatically increases. Nowhere is this more apparent than in the field of retinal dystrophies, with a vibrant synergy between molecular diagnosis and precise surgical delivery. The field has rapidly expanded from traditional gene augmentation approaches to allele-specific or mutation-specific suppression using CRISPR or antisense technology. The widespread use of large panels testing the approximately 271 known

genes associated with IRD has allowed seeing the landscape of genetic alterations in each patient, improving diagnostic yield, and mitigating against a false assumption of causality. This is especially important for gene therapy candidates, given the significant risks associated with the treatment. As individualized diagnostic and treatment algorithms continue to be developed, equally important will be early molecular diagnosis to facilitate treatment in a timely fashion before significant cellular degeneration has ensued.[56]

The youngest patient treated with subretinal gene delivery to the authors' knowledge was a 22-month-old girl with LCA at Children's Hospital Los Angeles. It is possible that children as young as 12 months could be treated with gene therapy if molecular diagnosis is established by then. The FDA label for voretigene neparvovec-rzyl recommends, however, against its use in children less than 12 months old because of ongoing retinal cell division, which could dilute the genetic material. In light of this, and the increased surgical complexity in very young infants, this seems a practical and scientifically sound lower limit at this time.

The prevalence of autosomal recessive IRDs is estimated to be 1 in 1380 individuals, with 5.5 million people expected to be affected globally.[57] Based on the current diagnostic yield of panel-based genetic testing in IRDs, 40% to 76% of these patients are expected to receive a genetic diagnosis.[4,58,59] Thus, the anticipated number of patients with IRDs who may undergo gene therapy in the future is substantial.

Advances in surgical delivery to the retina also have been an important driver of this blossoming trend in retinal gene therapy. Since developed by Maguire and others,[60] subretinal delivery seems effective and relatively safe, especially with ongoing improvements in vitreoretinal surgery visualization and instrumentation. Although the intravitreal approach represents an attractive outpatient procedure, the subretinal method currently appears to provide the best access to the photoreceptors and RPE, which are the target cell types for most dystrophies.

It is imperative to mention the growing need for a multidisciplinary, team approach when treating IRD patients. This includes having bioinformaticians and molecular pathologists in close communication with a retina specialist, who is in turn supported by genetic counselors, low vision specialists, operating room staff, and photographers familiar with advanced diagnostic testing, such as microperimetry, full-field scotopic sensitivity threshold testing, and ERG.

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DISCLOSURE

A. Nagiel serves as a consultant for REGENXBIO. N. Koulisis has nothing to disclose.

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