



# Gene therapy for inherited retinal diseases

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*Contributions:* (I) Conception and design: All authors; (II) Administrative support: SH Tsang; (III) Provision of study materials or patients: SH Tsang; (IV) Collection and assembly of data: All authors; (V) Manuscript writing: All authors; (VI) Final approval of manuscript: All authors.

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**Abstract:** Inherited retinal diseases (IRDs) are a genetically variable collection of devastating disorders that lead to significant visual impairment. Advances in genetic characterization over the past two decades have allowed identification of over 260 causative mutations associated with inherited retinal disorders. Thought to be incurable, gene supplementation therapy offers great promise in treating various forms of these blinding conditions. In gene replacement therapy, a disease-causing gene is replaced with a functional copy of the gene. These therapies are designed to slow disease progression and hopefully restore visual function. Gene therapies are typically delivered to target retinal cells by subretinal (SR) or intravitreal (IVT) injection. The historic Food and Drug Administration (FDA) approval of voretigene neparvovec for RPE65-associated Leber's congenital amaurosis (LCA) spurred tremendous optimism surrounding retinal gene therapy for various other monogenic IRDs. Novel disease-causing mutations continue to be discovered annually, and targeted genetic therapy is now under development in clinical and preclinical models for many IRDs. Numerous clinical trials for other IRDs are ongoing or have recently completed. Disorders being targeted for genetic therapy include retinitis pigmentosa (RP), choroideremia (CHM), achromatopsia (ACHM), Leber's hereditary optic neuropathy, usher syndrome (USH), X-linked retinoschisis, and Stargardt disease. Here, we provide an update of completed, ongoing, and planned clinical trials using gene supplementation strategies for retinal degenerative disorders.

**Keywords:** Inherited retinal disease (IRD); gene therapy; clinical trial

Submitted Jun 15, 2020. Accepted for publication Nov 04, 2020.

doi: 10.21037/atm-20-4726

**View this article at:** <https://dx.doi.org/10.21037/atm-20-4726>

## Introduction

Immune-privileged, enclosed, and easily monitored, the retina has been thoroughly investigated over the past two decades for gene therapy interventions (1). Inherited retinal diseases (IRDs), a genetically heterogeneous group of ocular disorders, are a leading cause of irreversible severe visual dysfunction worldwide. Many potential targets are available, as over 260 disease-causing genes for monogenic IRDs have been identified (RetNet [www.sph.uth.edu](http://www.sph.uth.edu)). The historic Food and Drug Administration (FDA) approval of voretigene neparvovec (Luxturna) spurred tremendous

optimism surrounding retinal gene therapy for various other monogenic IRDs. Disorders being targeted for genetic therapy include retinitis pigmentosa (RP), Leber congenital amaurosis, choroideremia (CHM), achromatopsia (ACHM), Leber's hereditary optic neuropathy, usher syndrome (USH), X-linked retinoschisis, and Stargardt disease. Since IRDs typically affect both eyes, the fellow contralateral eye has often served as an internal control in initial safety trials (2).

Gene replacement therapy currently forms the basis of most active clinical trials for IRD. In gene replacement therapy, a wild type copy of the pathogenic gene is

introduced into target retinal cells of interest via viral or non-viral vectors. These therapies are designed to slow disease progression and hopefully restore visual function. While promising for loss of function recessive and X-linked disorders, specific gene mutations and dominant IRDs with gain of function mutations require different approaches such as gene suppression, antisense oligonucleotides, or genome editing (3,4). Outer retinal photoreceptor and retinal pigment epithelial (RPE) cells are the primary targets in gene therapy. Subretinal (SR) injections, the main delivery method used in gene therapy, allow direct access to photoreceptor and RPE cells (5). Intravitreal (IVT) injection, a safer and simpler alternative to SR injections, is typically used when inner retinal cells or more widespread retinal areas are targeted (5).

For gene therapy, a vehicle is needed to deliver the genetic material. Recombinant adeno-associated viral (rAAV) vectors are the primary vehicles being used to deliver wild type complementary DNA (cDNA) in gene augmentation trials for IRDs. Efficient with little immunogenicity, rAAV vectors provide long term transduction in various types of retinal cells (6). Limited carrying capacity of 4.7 kB is the main disadvantage of AAV based delivery (6). AAV2 is the most studied and used serotype in clinical trials. With less neutralizing antibodies against AAV8 compared to AAV2 in the general population, AAV8 is an alternative serotype being more commonly adopted in preclinical and clinical research to decrease chances of an immune response (7). Novel AAV vectors, including large capacity dual AAV vectors, may broaden the applications of IVT injections enabling transduction of outer retinal cells and larger transgenes (8). Lentiviruses (LVs), an alternative viral vector with a larger cargo carrying capacity, are able to safely transfer large genes of interest (9). Non-viral delivery systems, such as nanoparticle-based vectors, are also under development to potentially reduce inflammatory risks while carrying larger transgenes (10).

Currently, there are over 40 clinical trials listed on [www.clinicaltrials.gov](http://www.clinicaltrials.gov) investigating gene augmentation therapy for IRDs (Table 1). This article will provide an overview of recent clinical trials using gene supplementation strategies for retinal degenerative disorders.

### RPE-65-associated Leber's congenital amaurosis (LCA)

LCA, a progressive visual disorder with a prevalence of 1:50,000–100,000, often presents with profound vision loss

and nystagmus in childhood (11). Biallelic mutations in *RPE65*, an encoder of an essential isomerase of the retinoid visual cycle responsible for the conversion of all-trans-retinyl ester to 11-cis-retinol, account for 3–16% of LCA cases (12,13). *RPE65*-associated LCA was the first IRD to be explored for gene therapy.

To date, there have been five prospective phase I/II clinical trials and one randomized control (RCT) phase III trial investigating the effectiveness and safety of a single SR injection of an AAV serotype 2 vector containing the cDNA of *RPE65* (AAV2-hRPE65v2) for patients with confirmed biallelic *RPE65* mutations. Initial human clinical trials in patients with severe disease confirmed a favorable adverse effect (AE) profile with complications consistent with SR injection with efficacy was supported by preclinical studies (14,15). Contralateral administration to untreated second eye of patients previously exposed to AAV2-hRPE65v2 was demonstrated to be safe (16). Efficacy results have been variable with some treated eyes showing sustained visual improvement on at least one outcome measure at 1–3 years, while others returned to pre-injection outcomes (17–19). The variable results could be explained by differences in disease severity, interstudy differences with respect to study design, dosage, surgical procedure, and vector engineering.

The efficacy endpoints in these studies focus on functional vision as opposed to structural changes. Primary efficacy outcomes measures included performance on a novel multi-luminance mobility test (MLMT). Secondary endpoints were best-corrected visual acuity (BCVA), full-field light sensitivity threshold (FST) to blue flashes, visual field (VF) testing, contrast sensitivity, pupillary light reflex, and mobility testing. Compared to other measurements, FST is more useful in patients with poor fixation as it measures the lowest luminance perceived over an entire VF. Investigators have recommended pairing the MLMT with FST to overcome the potential ceiling effect of MLMT (20). Visual acuity (VA), a measure of cone-mediated function, is not as suitable for primarily rod-mediated disorders such as *RPE65*-associated LCA.

In the phase III RCT (NCT00999609), AAV2-hRPE65v2 was sequentially administered in 29 participants (20 intervention: 9 controls) with confirmed *RPE65*-associated retinal dystrophy (age range, 4–44 years) (21). Second procedure on fellow eye was performed 6–18 days after the first. There was a high degree of safety with only mild procedure-related AEs. Significant improvements in MLMT score, FST, and VFs were apparent in the treatment group compared to controls at the 1 year follow-

**Table 1** Completed and ongoing gene replacement trials for inherited retinal diseases

Disease	Target	Phase	Vector	Delivery	Sponsor	NCT number
LCA	RPE65	I/II	AAV2	SR	Spark Therapeutics	01208389
	RPE65	III	AAV2	SR	Spark Therapeutics	0099609
	RPE65	I/II	AAV2	SR	Spark Therapeutics	00516477
	RPE65	I/II	AAV5	SR	MeiraGTx UK II Ltd	02781480
	RPE65	I/II	AAV6	SR	MeiraGTx UK II Ltd	02946879
	RPE65	I/II	rAAV2	SR	UCL	00643747
	RPE65	I/II	rAAV2/4	SR	Nantes	01496040
	RPE65	I	rAAV2	SR	Hadassah	00821340
	RPE65	I/II	rAAV2	SR	AGTC	00749957
	RPE65	I	rAAV2	SR	UPenn	00481546
Achromatopsia	CNGA3	I/II	AAV2/8	SR	MeiraGTx UK II Ltd	03758404
	CNGA3	I/II	rAAV	SR	STZ eyetrial	02610582
	CNGA3	I/II	AAV2/8	SR	MeiraGTx UK II Ltd	03758404
	CNGB3	I/II	AAV2/8	SR	MeiraGTx UK II Ltd	03001310
	CNGB3	I/II	rAAVtYF	SR	AGTC	02599922
Choroideremia	REP1	II	rAAV2	SR	STZ eyetrial	02671539
	REP1	I/II	rAAV2	SR	Ian M. MacDonald	02077361
	REP1	II	AAV2	SR	Byron Lam	02553135
	REP1	I/II	rAAV2	SR	University of Oxford	01461213
	REP1	II	AAV2	SR	NightstarRx Ltd	03507686
	REP1	III	AAV2	SR	NightstarRx Ltd	03496012
	REP1	II	AAV2	SR	UCL	02407678
	REP1	I/II	AAV2-hCHM	SR	Spark Therapeutics	02341807
Stargardt disease	ABCA4	I/II	Lentivirus	SR	Sanofi	01367444
Usher type 1B	MYO7A	I/II	Lentivirus	SR	Sanofi	01505062
XLRP	RPGR	I/II	AAV2/5	SR	MeiraGTx UK II Ltd	03252847
	RPGR	I/II	rAAV2tYF	SR	AGTC	03316560
	RPGR	I/II	AAV8	SR	NightstarRx Ltd	03116113
Retinitis pigmentosa	MERTK	I	rAAV2	SR	Fowzan Alkuraya	01482195
	PDE6B	I/II	AAV2/5	SR	Horama S.A.	03328130
	RLBP1	I/II	rAAV8	SR	Novartis Pharmaceuticals	03374657
LHON	ND4	II/III	rAAV2	IVT	Huazhong	03153293
	ND4	I/II	rAAV2	IVT	Huazhong	01267422
	ND4	I	AAV2	IVT	Huazhong	02161380
	ND4	III	rAAV2	IVT	GenSight Biologics	02652780

Table 1 (continued)

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Disease	Target	Phase	Vector	Delivery	Sponsor	NCT number
	ND4	III	rAAV2	IVT	GenSight Biologics	02652767
	ND4	III	rAAV2	IVT	GenSight Biologics	03293524
	ND4	I/II	rAAV2	IVT	GenSight Biologics	02064569
XLRS	RS1	I/III	rAAV2tYF	IVT	AGTC	02416622
	RS1	I/II	AAV8	IVT	NEI	02317887

LCA, Leber congenital amaurosis; XLRP, X-linked retinitis pigmentosa; LHON, Leber hereditary optic neuropathy; XLRS, X-linked retinoschisis; AAV, adeno-associated virus; rAAV, recombinant adeno-associated virus; SR, subretinal; IVT, intravitreal; UPenn, University of Pennsylvania; Nantes, Nantes University Hospital; Hadassah, Hadassah Medical Organization; AGTC, Applied Genetic Technologies Corp.; UCL, University College London; Huazhong, Huazhong University of Science and Technology; NEI, National Eye Institute.

up signifying partial rescue of photoreceptor function (21). From the open-label phase I follow-on study and the open-label, phase III RCT trial that Maguire and associates conducted, a substantial benefit was apparent by 30 days and sustained at 4 years (20). Notably, remodeling of visual cortex connections ipsilateral to treated eye was observed on neuroimaging technology (22).

In December 2017, following the successful randomized, controlled, open-label, phase III trial, Luxturna (voretigene neparvovec-rzyl, VN, Spark Therapeutics, Philadelphia, PA, USA) became the first ocular gene therapy approved by the FDA for *RPE65*-associated retinal dystrophy (21,23). Voretigene neparvovec later became approved by all members of the European Union in 2018.

A recent systemic review from the five prospective and one RCT *RPE65*-LCA gene therapy trials demonstrated that visual function outcomes improvements last only up to two years post-treatment (24). However, BCVA and FST were the only visual function outcomes that could be analyzed between studies (24). Other measures of visual function, such as MLMT and VF testing may show sustained improvement beyond 2 years. While prior safety analyses found a favorable AE profile, they found central retinal thickness thinning in treated eyes compared to untreated eyes 2–3 years following intervention (24). Nevertheless, it is difficult to make generalizations in these studies that had variable trial designs.

Time of genetic intervention initiation is an important consideration for future trials. Despite treatment, advanced staged LCA patients continue to show retinal degeneration suggesting initiation of therapy at earlier stages before irreversible degeneration is important (25). There may be

a threshold point past which target cells can no longer be rescued. In an initial phase I study, children showed the greatest gains on functional assessments (26). However, the oldest participants in the phase III trial showed similar improvements relative to the other patients (21). As retinal degeneration continues to slowly advance following gene therapy, adjunct therapies may be needed (25,27).

## ACHM

Patients with ACHM, a congenital autosomal recessive cone dysfunction disorder, present with early-onset pendular nystagmus, decreased VA, photosensitivity, and impaired color perception. Of the six phototransduction genes (*CNGA3*, *CNGB3*, *GNAT2*, *PDE6C*, *PDE6H*, and *ATF6*) known to be implicated in ACHM, loss-of-function mutations in *CNGA3* and *CNGB3* account for over 70% of ACHM cases (28,29). In cone photoreceptors, *CNGA3* and *CNGB3* encode the  $\alpha$ - and  $\beta$ -subunits of the cyclic nucleotide-gated (CNG) cation channel (30,31).

A recent nonrandomized clinical trial investigated the safety profile and efficacy of SR administration of an AAV8 vector encoding *CNGA3* (AAV8.CNGA3) in the worse eye of adult ACHM patients with biallelic pathogenic *CNGA3* mutations (NCT02610582) (32). Over a 1-year period, the treatment was well tolerated in the 9 treated patients with no serious adverse events observed. All AEs were mild expected complications of delivery method. Cone function recovery was observed in the treated eyes as demonstrated by improvements in color vision, VA, and contrast sensitivity (32).

There have been conflicting reports on the length of

the therapeutic window available for initiating treatment as some studies have demonstrated greater cone preservation at a young age while other structural studies showing no association between structural and functional changes with age (33-35). Supported by preclinical data, Fischer *et al.* hypothesized that gene therapy intervention for ACHM may be more effective at an earlier age when there is enhanced plasticity of the visual cortex (32,36). The recent clinical trial demonstrated that visual improvement could occur even in adult patients with advanced ACHM. Thus, there are 4 open-labeled phase I/II AAV gene therapy trials actively recruiting adults and children with *CNGA3* and *CNGB3* related ACHM (NCT03278873, NCT03758404, NCT02935517, NCT02599922). As some ACHM patients present with poor fixation and photophobia, reliable functional and structural test performance may be difficult to obtain in some study participants. To accurately assess therapeutic efficacy and guide future trials, cautious selection, and characterization of candidates prior to treatment may be essential in ensuring accurate analysis of visual function tests.

## CHM

CHM, an X-linked recessive disorder, is caused by mutations or deletions in the gene, *CHM*, an encoder of the intracellular trafficker Rab escort protein-1 (REP1) (37). In CHM, visual dysfunction begins in adolescence with night blindness and slowly progressive peripheral vision loss, cumulating in legal blindness by age 50 (38). Central photoreceptors do not degenerate until advanced CHM, prolonging the therapeutic window (39).

At 6 months, initial phase I/II trial results of the effects of SR *AAV2-REP1* gene therapy were promising with mild adverse events and improvements in BCVA in some of the treated eyes compared to contralateral untreated eyes (40). This improvement was sustained 3.5 years post-treatment in the two treated eyes with the most advanced disease (41). The other 4 patients had limited room for improvement as they already had good VA at baseline, but 3 of them maintained their baseline VA at 3.5 years of follow-up. Retinal sensitivity was notably improved in the patients that had little change in their VA (40).

Full results of this trial at the 2-year endpoint were recently reported (NCT01461213) (42). There were a total of 14 end-stage CHM patients that received either a low- or high-dose of AAV2-REP1 vector via SR injection. VA improved similarly in both low dose and high dose groups.

However, the cohort of patients in the study had advanced CHM limiting any concrete determination about what dose will be suitable for patients with better baseline macular function (42). Despite initial improvement, mean retinal sensitivity was not maintained over the 2-year follow-up.

Although VA is a useful functional outcome measure, VA decline is typically not observed until late stages of CHM (43). Alternative primary disease biomarkers will be necessary to employ in younger patients with earlier stage of CHM. Retinal sensitivity, as measured by microperimetry, may be a more useful early marker of efficacy, especially for patients who are treated early in the disease course. However, caution should be exercised when evaluating microperimetry results as test-retest variation has been demonstrated to be highly variable (44).

An additional phase I trial with a longer follow-up of 2 years and higher dose included ancillary structural and functional outcome measures (45). Improvements in BCVA and microperimetry observed in this study were similar to those observed in the first trial by Maclaren and associates. However, one untreated eye also showed significant improvement in BCVA raising additional queries over whether VA is a useful efficacy measure in CHM gene therapy trials (45). Area of residual autofluorescence (AF) on short-wavelength fundus autofluorescence (SW-FAF) imaging, known to decrease with disease severity, declined at the same rate between treated and control eyes (45,46).

There are 4 active or completed phase II studies evaluating AAV2-REP1 efficacy and safety in CHM patients. Two-year results from a phase II high-dose trial of 6 CHM patients treated with subfoveal injection of AAV2-REP1 were recently published (NCT02553135) (47). Consistent with phase I results, they reported a favorable safety profile as well as a sustained improvement in BCVA in the treated eyes. The untreated eyes showed no significant improvement in BCVA, contrary to the study by Dimopoulos *et al.* (45). No significant difference between treated and non-treated eyes were observed on microperimetry, FAF, or SD-OCT. Due to variable patterns of preserved AF, additional region-specific metrics may be necessary to elucidate therapeutic benefit in eyes with CHM (48). The patients enrolled in this trial had advanced CHM with more choroidal adhesions limiting the vector area of distribution (47). In mid-to advanced-stage CHM, therapy is directed towards remaining fragile islands of preserved RPE that maintain VA. As disease progression is relatively slower in the first decade of life, the authors suggested that initiating treatment in earlier stages of the

disease might increase vector exposure and resultant efficacy (47,49). So far, SR approach has been employed to target cells in the areas of preserved RPE. In early-stage CHM, with most of the retina unimpaired, some have suggested using an IVT approach to minimize risks of VA acuity loss due to trauma of SR injection from iatrogenic foveal detachment (5).

Additional 24-month data from a phase II open-label randomized trial (NCT02671539) of 6 subjects showed maintenance or gains in VA acuity over the 2-year period (50). However, there was no statistically significant difference in average BCVA change over time between treated and control eyes, most likely due to the limited sample size. Consistent with other trials, patients with more advanced CHM had greater improvements of BCVA (50).

A meta-analysis of 4 completed phase I/II AAV2-REP1 clinical trials validated safety and demonstrated BCVA maintenance or improvement in the study eye compared to control but no statistical differences were noted on structural measures (51).

As there is some inter-ocular asymmetry in progression rate, particularly in later stages, future trials for efficacy should be a randomized, masked, sham-controlled study (52). To this point, a randomized phase III trial of 169 patients is underway to validate therapeutic effectiveness (NCT03496012) (53). There will be three treatment groups: no treatment, low dose, and high dose. BCVA at 12 months is the main outcome measure, while secondary outcomes measures include changes in AF, ellipsoid zone change on OCT, microperimetry sensitivity, contrast sensitivity, color vision, and reading performance.

### Stargardt disease

Autosomal recessive Stargardt disease (STGD1) is caused by mutations in *ABCA4*, an encoder of a photoreceptor ATP-binding cassette (ABC) transporter (54). Typically diagnosed in adolescence, *ABCA4*-associated retinopathy causes progressive central or pericentral VF loss.

The size of *ABCA4* exceeds traditional AAV cargo capacity requiring alternative lentiviral, dual AAV, or non-viral vectors (55). The first phase I/II study of IVT delivery of an Equine Infectious Anemia Virus (EIAV) based lentiviral vector carrying *ABCA4* (SAR422459) in patients with STGD was terminated by Sanofi due review of clinical development plans and priorities (NCT01367444). The sponsor, Sanofi, is currently running a second similar trial investigating the long-term efficacy and

safety of SAR422459 in 27 participants (NCT01736592). Preliminary results at an ARVO meeting in 2017 reported favorable tolerability (56). Human clinical trials using AAV dual vector and non-viral nanoparticle systems are on the horizon as promising preclinical studies have effectively and safely delivered *ABCA4* transgenes using the novel platforms (57,58).

### USH

USH is a genetically heterogeneous group of autosomal recessive deafness-blindness syndromes characterized by RP, sensorineural hearing loss, and potential vestibular dysfunction. Hearing loss may be managed with cochlear implants, but the visual dysfunction has no available cure. USH is divided into three clinically distinct types based on time course and degree of multi-sensory loss: USH1, USH2, and USH3. Patients with Usher syndrome 1 (USH1), the most severe subtype, present at birth with extreme hearing loss, vestibular difficulty, and slowly progressive early-onset RP. The six disease-causing genes associated with USH1 are *USH1C* (harmonin), *CDH23* (cadherin 23), *PCDH15* (protocadherin 15), *USH1G* (sans), *CIB2* (calcium and integrin binding family member 2), and *MYO7A* (myosin VIIA) (59-64). Mutations in *MYO7A* are linked to the most common form of USH1, Usher 1B (65). Gene replacement therapy may be initiated in USH1B patients early in the disease course as they are often diagnosed before structurally apparent retinal degeneration. *MYO7A* is a large gene beyond the carrying capacity of AAV vectors, necessitating the use of lentiviruses and dual AAVs (66). Traditional AAVs only have the carrying capacity for 5 of USH disease-causing genes: *CIB2*, *CLRN*, *WHRN*, *USH1C*, and *USH1G*.

The first human gene therapy replacement trial for *MYO7A*-linked USH1B investigated the safety of a lentiviral vector delivered via SR injection. Sanofi, the sponsor of the study, ceased the exploratory study due to review of clinical development plans and priorities. Currently, Sanofi is running two trials to investigate the tolerability of SR administration of an EIAV-based lentiviral vector carrying *MYO7A* (SAR421869) for patients affected with USH1B (NCT02065011 and NCT01505062). Preliminary findings in 2015 demonstrated no serious AEs (67).

### RP

RP encompasses a visually devastating collection of

progressive rod-cone dystrophies responsible for a majority of inherited retinal disorders (IRDs). Symptoms typically progress from night blindness to constricting daytime peripheral VF loss and eventual total blindness after loss of central vision (68). Over 67 causative genes with many disease-causing variants have been identified in association with RP (RetNet [www.sph.uth.edu](http://www.sph.uth.edu)). Several sponsors and investigators have attempted to correct different forms of RP with AAV mediated gene augmentation therapy.

### **XLRP-RPGR**

X-linked retinitis pigmentosa (XLRP), a severe early-onset form of RP, is most commonly caused by mutations in the RP GTPase regulator (*RPGR*) gene (69). Males will typically present in early childhood with progressive nyctalopia and narrowing of peripheral VFs that result in legal blindness by the fourth decade (70). While female carriers have a variable phenotype, they typically do not experience visual loss. The *RPGR* protein, encoded by the *RPGR* gene, is involved in ciliary transport and critical in maintaining photoreceptor integrity (71). Accounting for a considerable percentage of overall RP cases, and easily assessed over a relatively short time period, genetic interventions for *RPGR*-associated XLRP have been heavily pursued (72).

Cehajic-Kapetanovic *et al.* recently published initial 6-month results of the first phase I/II clinical trial assessing the safety and efficacy of a subretinally administered AAV8 vector encoding *RPGR* (AAV8-*coRPGR*) in 18 male patients with XLRP (NCT03116113) (73). A codon-optimized *RPGR* sequence was employed to stabilize sequence integrity and increase transgene expression (74). All vector doses were well tolerated with no serious AEs. At 6 months following treatment, subjects who received mid-doses of the vector demonstrated sustained gains of VFs and retinal sensitivity on microperimetry. Structurally, an increase in outer nuclear layer (ONL) thickness on OCT was observed in the treated eyes. Results from the 2-year follow up will help establish the longer term safety and efficacy of AAV8-*coRPGR* gene therapy.

There are two additional ongoing nonrandomized dose-escalation phase I/II clinical trials evaluating the safety and efficacy of SR gene therapy for male patients with *RPGR*-associated XLRP. All *RPGR* gene therapy trials use rhodopsin kinase (RK or GRK1) promoter, but the following investigations are using a different combination of viral vector subtypes and coding sequences from the study that was recently published. One clinical trial is evaluating

the safety and efficacy of a rAAV2tYF-GRK1-*RPGR* mutations (NCT03316560), while another is assessing the use of AAV2-RPGR (NCT03252847).

### **MERTK-, RLBP1- and PDE6B-associated RP**

*MERTK*-associated RP, similarly to *RPE65*-associated LCA, involves dysfunction of the RPE with mutations in *MERTK* implicated in RPE phagocytosis of photoreceptor segments (75). A phase I dose-escalation trial assessed the SR administration of rAAV2-VMD2-h*MERTK* in 6 participants with *MERTK*-associated RP (NCT01482195) (76). Treatment was well tolerated over the 2-year period with no signs of serious ocular or systemic AEs. Three patients showed improvements in BCVA, but only one patient maintained the visual gain at the 2-year follow-up. Both patients that had transient gains in BCVA developed bilateral cataracts, potentially confounding the therapeutic benefit. The authors hypothesized that patient selection might be critical in successful intervention as subjects with better function at baseline demonstrated greater improvements (76). While similar to other types of RP, there have been no extensive natural history studies to determine the rate of progression and gene-specific outcome measures. The investigators plan to follow the patients for an additional 13 years (76).

Following encouraging preclinical studies, human AAV mediated gene therapy trials have also begun for *RLBP1*- and *PDE6B*-associated RP using rAAV8 and rAAV2/5, respectively (NCT03374657, NCT03328130).

### **Leber hereditary optic neuropathy (LHON)**

LHON is the most common mitochondrial DNA disorder, typically presenting in young males as concurrent or consecutive loss of central vision bilaterally (77). Mitochondrial DNA (mtDNA) point mutations in the nicotinamide adenine dinucleotide dehydrogenase subunit 4 (ND4), a critical component of the respiratory chain, are responsible for a majority of LHON cases (78). Retinal ganglion cell damage and subsequent optic atrophy are induced from the increased generation of reactive oxygen species caused by a dysfunctional respiratory chain (78).

Multiple active and upcoming clinical trials are investigating the safety and efficacy of AAV mediated gene replacement with optimized allotopic expression for LHON patients carrying ND4 mutations (mt11778G→A) (79). As retinal ganglion cells are the primary target of therapy,

AAV vectors are delivered via IVT injections. The first LHON gene therapy trial assessed the safety, efficacy, and sustainability of IVT rAAV2-ND4 injections in 9 patients with mutations in ND4 (NCT01267422) (80). Eight patients received a single unilateral injection while one patient received an injection in the contralateral eye one year after initial treatment. Seven-year follow-up demonstrated gene therapy was safe with no reports of systemic or ocular AEs (81). At the 36-month follow-up, treated eyes showed both functional and structural improvements. Functional improvements were observed in BCVA and VFs in both the injected and uninjected eyes. While the retinal nerve fiber layer (RNFL) thicknesses continued to decrease in the fellow control eye, it was maintained at baseline values in the treated eyes (80). At the 7-year follow-up, 6 patients maintained their bilateral improvements in BCVA (81). Functional improvements were more robust in younger, recently diagnosed patients, suggesting initiation of therapy early in the disease course (81). The sponsor of this study, Huazhong University of Science and Technology, is conducting a larger phase II/III study with 142 subjects to confirm the promising safety, efficacy, and durability of rAAV2-ND4 gene therapy (NCT03153293). Initial 3-month results of this larger scale study looked at the baseline functional characteristics that correlated with visual recovery (82). Better baseline visual field index (VFI) and BCVA were found to be strong predictors of functional improvement (82).

In 14 LHON patients, Guy and associates conducted a 24-month study evaluating the efficacy and safety of IVT injection of AAV2 (Y444,500,730F)-P1ND4v2 (NCT02161380) (83). No dose-related toxicities were observed, with only mild adverse events reported. Similarly to Yuan *et al.*, they observed more substantial visual recovery in patients that had visual loss commence for less than 1 year before treatment (81,83). While some non-treated eyes demonstrated improvements in BCVA, the treated eyes had a significantly larger improvement compared to the control non-treated eye (83).

Over a 2-year phase I/II dose-escalation trial, GenSight Biologics demonstrated a favorable safety profile for single unilateral IVT administrations of rAAV2/2-ND4 (GS010) in participants carrying the ND4-G11778A mutation (84). GenSight Biologics recently released press releases on the 96-week results of two separate randomized, sham-controlled phase III AAV mediated clinical trials evaluating the efficacy GS010 (rAAV2/2-ND4) (85,86). The RESCUE trial (NCT02652767) included patients with

less than 6 months of visual loss, while the REVERSE trial (NCT02652780) treated subjects that were affected for 6 to 12 months. Sustained bilateral functional improvement in BCVA and contrast-sensitivity were observed in both trials (85,86). Given the encouraging results, the GenSight Biologics team is preparing for the regulatory approval process. A planned meta-analysis of the data will help inform the optimal therapeutic window for visual recovery and help direct future treatments. GenSight is additionally running a trial to evaluate the safety and efficacy of bilateral injections of GS010 in LHON patients (NCT03293524).

### X-linked juvenile retinoschisis (XLRS)

XLRS is a hereditary retinal degeneration affecting 1:5,000 to 1:20,000 young males (87). XLRS is caused by mutations in the gene retinoschisin 1 (*RS1*), an encoder of retinoschisin, a secretory protein essential for retinal organization and intracellular adhesion (88). Patients will typically present in early childhood with a slowly progressive loss of central vision that may be complicated by retinal detachment and vitreous hemorrhage as they age. Clinically, XLRS is typically characterized by bilateral foveal splitting (schisis) with small cystoid spaces arranged in a spoke-wheel pattern on funduscopy (89). While carbonic anhydrase inhibitors and vitreoretinal surgery are being used in a subset of patients to treat disease-related complications, gene-based therapy is a promising treatment for restoring retinal integrity.

There are currently two ongoing gene replacement trials for XLRS. Given the retinal vulnerability and propensity for developing retinal detachments, IVT injections are favored over SR for delivering the viral agent in XLRS studies. Preclinical animal studies demonstrated the internal limiting membrane (ILM), a major transduction barrier to effective gene expression from the vitreous, is weakened in XLRS, permitting the use of IVT injections (90,91). Cukras *et al.* recently detailed the initial findings of a National Eye Institute (NEI) sponsored phase I/IIa dose-escalation trial evaluating the IVT administration of AAV8-*RS1* gene therapy in 9 male XLRS patients (NCT02317887) (90). Overall, the procedure and vector were well tolerated at month 18 of follow-up (90). Of the functional outcome measures used to assess efficacy (BCVA, MP1 retinal sensitivity, and ERG response), there were no clear visual gains compared to baseline. Structurally on OCT, a temporary cystic space closure was observed in one treated eye but not the contralateral control eye (90). While



there was no functional improvement during this period of closure, the limited effect suggests potential therapeutic benefit (90).

Using a different vector subtype, Applied Genetic Technologies Corporation (AGTC), is conducting a phase I/II dose-escalation trial of rAAV2tYF-CB-hRS1 therapy delivered via IVT injection (NCT02416622). In a preclinical study, AAV2 was found to transduce ganglion cells following IVT injections more efficiently compared to AAV8 (92). A press release from AGTC describing 6-month results reported a favorable safety profile but no functional improvement (93). As XLRS is a slowly progressive disease, longer follow-ups of these 2 trials are likely necessary to observe functional improvement.

## Conclusions

With the FDA approval of voretigene neparvovec (Luxturna) for *RPE65*-associated retinal dystrophy and encouraging clinical trials for other IRDs, many clinicians and patients are hopeful that a gene therapy for numerous other visually debilitating and ‘incurable’ IRDs is on the horizon. Novel disease-causing mutations continue to be discovered annually, and targeted genetic therapy is now under development in clinical and preclinical models for many IRDs.

Adjusting patient expectations undergoing gene therapy will be important moving forward. Successful therapy may result in halting progression and preserving baseline function and structure. Gene-specific natural history and outcome measure studies should be emphasized in the future to guide future therapies. Comprehensive analysis of baseline characteristics that lead to better functional and structural gains following gene therapy will influence and improve patient risk-benefit discussions. Continued evolution of assessment modalities, vectors, and delivery methods will hopefully help overcome many of the remaining challenges to effective and durable gene therapy for IRDs.

## Acknowledgments

**Funding:** This work was supported by the Jonas Children’s Vision Care and Bernard & Shirlee Brown Glaucoma Laboratory, the National Institutes of Health (P30EY019007, R01EY018213, R01EY024698, R01EY026682, R21AG050437), National Cancer Institute Core (5P30CA013696), Foundation Fighting

Blindness (TA-NMT-0116-0692-COLU), New York State (SDHDOH01-C32590GG-3450000), the Research to Prevent Blindness (RPB) PhysicianScientist Award, and unrestricted funds from RPB, New York, NY, USA.

## Footnote

**Provenance and Peer Review:** This article was commissioned by the Guest Editor (Susanna S. Park) for the series “Novel Tools and Therapies for Ocular Regeneration” published in *Annals of Translational Medicine*. The article has undergone external peer review.

**Peer Review File:** Available at <https://dx.doi.org/10.21037/atm-20-4726>

**Conflicts of Interest:** All authors have completed the ICMJE uniform disclosure form (available at <https://dx.doi.org/10.21037/atm-20-4726>). The series “Novel Tools and Therapies for Ocular Regeneration” was commissioned by the editorial office without any funding or sponsorship. The authors have no other conflicts of interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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**Cite this article as:** Nuzbrokh Y, Ragi SD, Tsang SH. Gene therapy for inherited retinal diseases. *Ann Transl Med* 2021;9(15):1278. doi: 10.21037/atm-20-4726