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REVIEW

Voretigene Neparvovec in Retinal Diseases: A Review of the Current Clinical Evidence

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Abstract: Subretinal gene therapy trials began with the discovery of *RPE65* variants and their association with Leber congenital amaurosis. The RPE65 protein is critical for the normal functioning of the visual phototransduction cascade. *RPE65* gene knockout animal models were developed and showed similar diseased phenotypes to their human counterparts. Proof of concept studies were carried out in these animal models using subretinal *RPE65* gene replacement therapy, resulting in improvements in various visual function markers including electroretinograms, pupillary light responses, and object avoidance behaviors. Positive results in animal models led to Phase 1 human studies using adeno-associated viral vectors. Results in these initial human studies also showed positive impact on visual function and acceptable safety. A landmark Phase 3 study was then conducted by Spark Therapeutics using a dose of 1.5 $x10^{11}$ vector genomes after dose-escalation studies confirmed its efficacy and safety. Multi-luminance mobility testing was used to measure the primary efficacy endpoint due to its excellent reliability in detecting the progression of inherited retinal diseases. After the study met its primary endpoint, the Food and Drug Administration approved voretigene neparvovec (Luxturna®) for use in *RPE65*-associated inherited retinal diseases.

Keywords: gene therapy, inherited retinal diseases, Leber congenital amaurosis, Luxturna, RPE65, voretigene neparvovec, retinitis pigmentosa, retina

Introduction

Inherited retinal diseases (IRDs) are a heterogenous group of disorders characterized by varying degrees of functional vision loss and associated retinal degenerative changes. For the collective 270 gene mutations that have been identified in association with clinically diagnosed IRDs, the incidence is approximately 1 in 2000.[1,2] In most IRDs, the visual loss occurs early and can be profound, resulting in significant disability to the patient. The primary site of degeneration usually involves the photoreceptor and retinal pigment epithelium (RPE) complex. IRDs can be classified as either stationary, such as in congenital stationary night blindness (CSNB), or progressive, such as in retinitis pigmentosa (RP).[1] Leber congenital amaurosis (LCA) is one of the most severe types of progressive IRDs, presenting with significant functional vision decline within the first year of life.[3,4] This review will provide a brief overview on the *RPE65* gene mutation-related dystrophies and focus on the clinical evidence that led to the approval of voretigene neparvovec-rzyl, the first FDA (Food and Drug Administration)-approved gene replacement therapy in the United States and in the European Union.[5,6]

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The *RPE65* Gene

Gene Function

The *RPE65* gene encodes for a 65 kDa protein located primarily on the smooth endoplasmic reticulum of RPE cells.[7] Electroretinograms (ERGs) of biallelic knockout (*RPE65* -/-) mice demonstrated diminished or absent waveforms similar to what is seen in humans. Dark-adapted ERGs tended to be worse than light-adapted or flicker ERGs suggesting that rod function is more severely impacted than cone function. In the photoreceptors of these eyes, no detectable rhodopsin was found. In the RPE cells, there was an absence of 11-cis-retinol and an overaccumulation of all-trans-retinol.[8] In initial in vitro studies, human cells were transfected with the *RPE65* gene along with a *LRAT* coenzyme gene, followed by exposure to all-trans-retinol. This resulted in decreased all-trans-retinol levels and a dramatic increase in 11-cis-retinol levels, suggesting that the RPE65 protein plays a direct enzymatic role in the isomerization of 11-trans-retinol to 11-cis-retinol.[9,10]

Other studies suggest that a non-enzymatic role for the RPE65 protein may exist. One study noted that heterozygous (*RPE65* +/–) mice have a much larger drop in rod pigment recycling than would be expected compared to wild type (*RPE65* +/+) mice if the RPE65 protein was acting solely as an enzyme. This study also showed that when *RPE65* -/- mice were given oral 9-cis-retinal supplementation, a small amount of 11-cis-retinal could be detected. The author suggested that other proteins may be involved in the visual cycle pathway that eventually produces 11-cis-retinal. They concluded that the RPE65 protein could either have a dual function as both an isomerase enzyme and a structural protein or as an organizer protein involved in the distribution of retinyl esters within the RPE cells.[11] Currently, the exact function of the RPE65 protein is still debated, but what is known is that 11-cis-retinol is converted to 11-cis-retinal which is required by the photoreceptor outer segments in order to combine with opsin and produce the visual pigment that is responsible for detecting light and initiating the phototransduction cascade.[7]

Mutant *RPE65* Gene Phenotypes

Biallelic *RPE65* gene mutations can manifest clinically as Leber congenital amaurosis type 2 (LCA2) or as rare types of retinitis pigmentosa. The incidence of LCA is estimated to be around 2 to 3 per 100,000, and this condition accounts for 5% of all IRDs diagnosed. LCA2 is due to a mutation in the *RPE65* gene mapped to chromosome 1p31. Phenotypically, patients present in infancy with absent fixation, eye wandering, nyctalopia, nystagmus, and progressive vision loss.[3,12–17] Fundus examination may initially be normal, but ERG will show severely diminished or absent waveforms consistent with severe rod and cone dysfunction.[18] Over time, the dilated exam may show signs of degeneration presenting as retinal vascular attenuation, optic disc atrophy, peripheral pigmentary spicules, nummular pigmentation, peripheral yellow spots, or para-arteriolar pigmentary changes.[12] While over 100 mutations have been identified in RP, only 2% of autosomal recessive RP patients have mutations in the *RPE65* gene. Clinical presentation may vary, but most patients will present in early childhood with nyctalopia and peripheral vision loss that eventually progresses to central vision loss. On exam, diffuse pigmentary changes, arteriolar attenuation, and optic nerve pallor can be seen (Figure 1). A choroideremia-like picture has also been reported with an autosomal dominant type of RP associated with the *RPE65* gene mutation. On ERG, a reduction in a- and b-wave amplitudes is noted in the earlier stages of the disease, but these waveforms may completely extinguish in later stages of the disease.[19]

One study of over 200 patients diagnosed with either isolated RP, autosomal recessive RP, or LCA showed that 2% of the RP patients and 16% of the LCA patients had mutations in the *RPE65* gene. This suggests phenotypic heterogeneity within *RPE65* gene mutants. The authors clinically differentiated between RP and LCA by stating

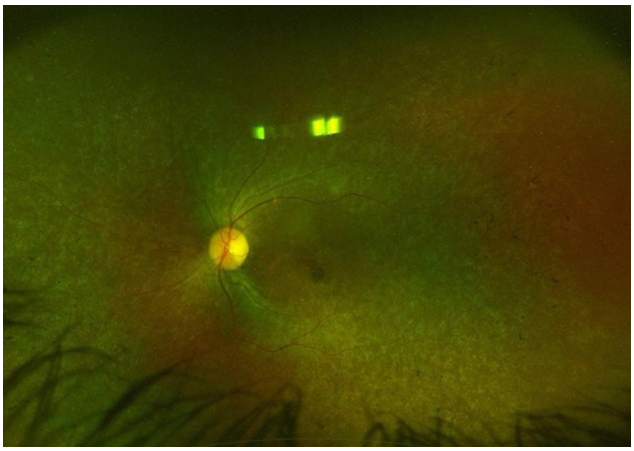

Figure 1 Fundus photograph of the left eye of a 33-year-old female with biallelic *RPE65* gene mutation demonstrating diffuse pigmentary changes, vascular attenuation, and temporal disc pallor.

that LCA patients were either born blind or developed severe vision loss within the first few months of life while RP patients showed good central vision within the first decade of life with eventual worsening afterwards.[20] This phenotypic heterogeneity could be explained by the variability in mutant protein function depending on the exact location of the mutation within the gene. Given the potential variability in phenotypic presentation for the same gene mutation and the increased accessibility of genetic testing, there has been a general shift from diagnosing IRDs by their phenotypic presentation towards identifying IRDs by their genotypic profile.

Gene Therapy and the Development of Voretigene Neparvovec

Immune Privilege and the Subretinal Space

The eye is an excellent site for gene therapy because of its immune privileged status and its compartmentalized structure that allows for targeted delivery of medication to intraocular cells. Immune privilege was originally described by a phenomenon known as anterior chamber-associated immune deviation (ACAID) where foreign antigens presented into the anterior chamber (AC) were observed to produce less of an immune response than expected. It was hypothesized that the AC had the ability to upregulate the expression of suppressive regulatory T cells towards foreign antigens.[21] This same concept was tested in the subretinal space by injecting P815 mastocytoma cells and ovalbumin acting as a soluble antigen into mice. Immune response was tested by seeing if a delayed-type hypersensitivity (DTH) reaction would occur after reinoculation of the P815 cells or ovalbumin behind the mice's ears. The ear challenge confirmed the lack of an antigen-specific DTH reaction.[22] Experiments like this proved that the subretinal space also exhibited immune deviation likely due to the presence of a blood-retinal barrier and local production of regulatory suppressor T cells. This unique immune property opened the pathway to exploring the use of viral vectors within the subretinal space.

Viral Vector Development

Successful gene replacement relies on the ability of a vector to safely insert itself into the targeted cell followed by implantation of the packaged genetic material. This must then lead to further translation of the gene into functional proteins without causing insertional mutagenesis, oncogenesis, or an exorbitant host immune response.[23] One of the most commonly used vectors for gene therapy is the adeno-associated viral (AAV) vector. AAVs are small (25 nm) non-enveloped single-stranded DNA viruses belonging to the Parvoviridae family. Because they require helper viruses to replicate, they are considered non-pathogenic. Another advantage is that AAV vector DNA remains episomal and does not integrate into host DNA thereby decreasing the risk for oncogenesis or mutagenesis. Disadvantages include a small packing capacity (can only hold up to 4.5 kB of complementary DNA), and the inability of DNA expression to perpetuate in dividing cells.[24] There are over 100 AAV variants with varying degrees of tropism for different human tissue types. Serotypes 1 through 9 all have affinity for ocular tissue and efficiently transduce RPE cells. AAV is fairly ubiquitous within humans with over 70% of the population showing positive antibodies (Ab) to AAV1 and AAV2 antigens.[25] Many factors play a role in determining the efficiency of the DNA expression, including the type of promoter used, the addition of enhancer sequences, and vector capsid components.[26,27] Capsid proteins can be modified to improve transduction, cell targeting, and counter pre-existing immunity.

The AAV2 vector used in the *RPE65* gene therapy trials transduces mainly RPE cells and, to a lesser degree, photoreceptors. This transduction and transgenic gene expression occur by 2 to 4 weeks following subretinal inoculation.[28] This property of the AAV2 vector to preferentially target RPE cells coupled with the fact that the *RPE65* gene is mainly expressed in RPE cells and also small enough to be packaged into the AAV2 vector all contributed to the success observed in later animal and human trials.

Pre-Clinical Animal Studies

Unlike many other IRDs, *RPE65*-associated LCA had the advantage of being established in small and large animal models which provided a platform for forthcoming gene replacement therapy experiments. RPE65 -/- mice and dogs were the first animal models tested.[29,30] In 2001, the first report of a subretinal gene therapy trial in a canine model was released. The study used an AAV vector to deliver functional *RPE65* genes into *RPE65* -/- dogs. Both cone and rod ERGs improved after subretinal injections, but not after an intravitreal injection. Pupillary light response (PLR), object avoidance, and visual evoked potential also improved. Enucleation of one of the eyes that received

a subretinal injection confirmed the expression of transgenic wild *RPE65* gene by PCR testing.[31] A follow-up study showed that improvements were stable at 3 to 5 years without significant inflammation or diminished ERG responses.[32] Additional mice studies also showed improvements in both cone and rod ERG waveforms. Furthermore, structural improvements were seen with respect to a decrease in lipid inclusions within the RPE cell, although this did not appear to halt photoreceptor degeneration.[33]

Further dose escalation trials were done in mice, dogs, and eventually monkeys to establish a potentially safe and efficacious starting dose for human trials. An initial study in dogs evaluated a wide range of vector genomes (vg) from 1.5 $x10^8$ vg to 4.5 $x10^{12}$ vg. Improvements in ERG amplitudes were noted in most dogs at doses of 1.5 $x10^{11}$ vg or higher. Transient ocular inflammation and variable mild serum Ab responses to the AAV antigen were noted in some animals, but no major systemic sequalae were noted. Later studies in macaque monkeys showed that dose levels of 1.5 $x10^{12}$ vg and 4.5 $x10^{12}$ vg were well-tolerated without evidence of retinal toxicity.[34] These studies helped pave the way for subsequent human trials.

Pre-Treatment Considerations in Humans

Prior to undergoing surgery for gene replacement therapy, the integrity of the photoreceptor layer must be evaluated. The formal indication for voretigene neparvovec use requires the presence of "viable retinal cells". Visual function loss in *RPE65*-associated IRD is comprised of two components: a visual cycle blockade that is reversible with gene therapy and a cellular degenerative process which is unlikely to be reversible in its end stages. Experiments in older mice showed that the therapeutic responders on average had 4 rows of photoreceptor nuclei in histological sectioning compared to therapeutic failures which only had 1 row.[35] Because of the implausibility of actual retinal tissue sampling, optical coherence tomography (OCT) has been used as a surrogate marker for photoreceptor health (Figure 2). Even so, OCT studies on human *RPE65* gene mutants have not shown a strong correlation between the degree of visual function loss or age and OCT characteristics such as outer nuclear layer (ONL) thickness. Therefore, deciding whether a patient's photoreceptor layer has sufficient viable cells to successfully respond to gene therapy is left to the discretion of the treating provider.[35,36]

Phase I/II Human Studies

In 2008, the results from the first human gene therapy trials were published from 3 separate groups. Each group recruited 3 patients (age 16–24 years) and gave unilateral subretinal injections of the *RPE65* gene carried by AAV2 vectors to the worse-seeing eye. Despite differences in baseline visual acuity (VA), treatment dosages (eg, 1.5 $x10^{10}$ vg, 5.96 $x10^{10}$ vg, 1 $x10^{11}$ vg), regulatory elements (eg, promoters, enhancers), surgical techniques (eg, gas displacement of subretinal bleb in one study) and injection locations (eg, 6 fovea-involving, 3 extramacular) between the studies, improvements in various visual function markers were observed in all three studies. No serious systemic immune-related side effects were seen.[37–39] These encouraging safety and efficacy results spurred further dose-escalation and sequential fellow eye treatment studies.

Maguire et al reported the first dose-escalation study in 2009 involving 12 patients (age 8–44 years) subdivided into low (1 $x10^{10}$ vg), medium (4.8 $x10^{10}$ vg) and high (1.5 $x10^{11}$ vg) dose treatment groups. Again, unilateral treatments were given to the worse-seeing eye. VA gains were seen in 7 patients (3 low, 3 medium, and 1 high dose) with no association to age or dosage received. Goldmann visual field (GVF) improved in all 12 and was associated

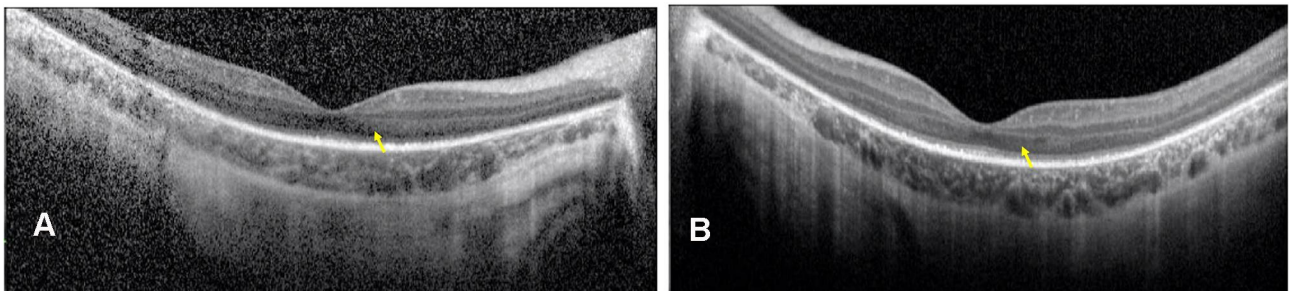

Figure 2 Optical coherence tomography images of the right (**A**) and left (**B**) macula of a 6-year-old boy with biallelic *RPE65* gene mutation. Notice the diffuse loss of the ellipsoid zone except for a small subfoveal island in both eyes. Also note the marked atrophy of the outer nuclear layer (arrows) which slightly increases in thickness in the parafoveal and foveal regions.

with subretinal injection location as well as the amount of quantifiable healthy outer retina. Five of 7 patients improved in dark-adapted full-field sensitivity threshold (FST) testing, but no improvements were noted on ERG, unlike what was observed in animal models. No systemic toxicity or adverse immunologic responses were seen despite transient elevations in Ab against AAV2 vector antigens in the tears and blood of some participants.[40] A follow-up study showed that the improvements were stable out to 3 years. Analysis of patients with sustained VA gains and improved fixation on microperimetry suggested that the improvements were likely due to decreases in nystagmus which was observed in both treated and untreated eyes.[41] A functional MRI (fMRI) study on 10 of the 12 patients showed improved signaling in the occipital lobe corresponding to the subretinal treatment location.[42]

Additional dose-escalation trials followed. Jacobson et al showed similar improvements in dark-adapted FST in all 15 subjects studied. Blue light stimulus was used for FST to show that the improvements were largely rod-mediated. About half the patients improved on mobility testing in low light levels when using the treated eye, but not with the fellow control eye.[43] Three patients were recruited for a long-term follow-up study. Surprisingly, results showed that the dark-adapted retinal sensitivity maps initially expanded in size and peaked in the first 1 to 3 years after treatment, but then slowly contracted in subsequent years. ONL thickness scans showed gradual but persistent declines from baseline to years 4 to 6. This could be explained by either initial photoreceptor rescue secondary to restoration of the visual cycle from gene therapy with subsequent decline in transgenic expression over time; or an RPE65 protein-independent progressive photoreceptor degeneration; or a combination of both.[44] Bainbridge et al employed the highest dosing regimens (1×10^{11} vg, 1×10^{12} vg). This study of 12 patients (age 6–23 years) showed similar initial improvements in dark-adapted perimetry, microperimetry, and navigation, especially in the higher dose group. However, the higher dose group also showed a higher incidence of intraocular inflammation with 1 patient developing focal pigmentary changes in the macula resulting in a 15 letter VA loss. Again, no correlation was seen between treatment success and age. In fact, the older patients (age 17–23 years) had greater initial improvements. Similar to earlier studies, improvements waned beyond the first year post-treatment causing focus to shift from increasing the vg dosage to optimizing the efficacy of the vector construct.[45] More recently, Weleber et al published results at 2 years showing at least 1 visual function improvement in 9 out of 12 patients (age 6–39 years) using medium-dose regimens (1.8×10^{11} vg, 6×10^{11} vg) which were sustained at 5 years. While the effects were not dose-dependent, they did find that younger patients (age 6–11 years) tended to have greater improvements. The visual function improvements corroborated the previously observed correlation between dampening of nystagmus and improvement in foveal fixation, explaining why both treated and untreated eyes improved. Of note, results could have been skewed because of very poor baseline vision in the older group (age 28–39) with the majority of these patients having VA of counting fingers or worse along with severely reduced baseline visual fields (VF).[46,47]

Gene therapy retreatments to the contralateral eye of previously-treated canines and non-human primates showed that sequential treatments were safe. Re-exposure to subretinal gene therapy in the fellow eye did not incite an exaggerated immune response or a decrease in RPE65 protein expression. Early human contralateral eye studies demonstrated efficacy through improvements in FST, obstacle avoidance, and bilateral cortical activation on fMRI.[48] Results from a more recent study evaluating sequential contralateral treatments showed FST improvements for both rod- and cone-directed stimuli, although the rod response appeared more robust. Improvements in cortical activation on fMRI and navigational performance in progressively lower light levels were also noted. Previous AAV2 serum Ab positivity did not diminish the visual function gains.[49] Given the positive results from these dose-escalation and contralateral redosing studies (Table 1), Spark Therapeutics proceeded to sponsor a phase 3 clinical trial.

Novel Primary Endpoint in IRD Studies

Before initiating a phase 3 human gene therapy trial, an appropriate primary efficacy endpoint needed to be established. Because affected patients typically have very poor baseline VA, using VA as the primary outcome measure is not ideal. Since rod dysfunction is more prominent in the early stages of *RPE65*-associated dystrophy, it is reasonable to assume that navigational abilities in lower light settings will initially be more significantly affected. Chung et al designed a multi-luminance mobility test (MLMT) in an effort to create a single quantifiable test that could be used to assess visual function changes in patients with rod-dominant IRDs. The

Table 1 Summary of the Major Human Clinical Trials Evaluating *RPE65* Gene Therapy

Study	Trial Phase	Methods	Main Results
Maguire et al 2008[37]	Phase I	Patient population • 3 patients • Unilateral worse eye treated • 19 to 26 years old Dose • 1.5 x10^{10} vg Follow-up • 1.25 to 4.75 months	Safety • No major immune-related adverse events • One patient developed a macular hole Visual function outcomes • All patients improved in VA, GVF, PLR, and subjective visual function in dim lighting; all had decreased monocular and binocular nystagmus • One patient improved in mobility testing
Hauswirth et al 2008[38]	Phase I	Patients population • 3 patients • Unilateral worse eye treated • 21 to 24 years old Dose • 5.96 x10^{10} vg Follow-up • 3 months	Safety • No major immune-related adverse events • One patient developed foveal thinning Visual function outcomes • 2 patients improved in dark-adapted FST • All patients reported subjective visual improvement in dim lighting
Bainbridge et al 2008[39]	Phase I	Patient population • 3 patients • Unilateral worse eye treated • 17 to 23 years old Dose • 1 x10^{11} vg Follow-up • 6 to 12 months	Safety • No major immune-related adverse events Visual function outcomes • One patient improved in microperimetry • One patient improved in dark-adapted perimetry • One patient improved in mobility testing • One patient reported subjective visual improvement in dim lighting
Maguire et al 2009[40]	Phase I/2 dose-escalation	Patient population • 12 patients • Unilateral, worse eye treated • 8 to 44 years old Doses • 1.5 x10^{10} vg • 4.8 x10^{10} vg • 1.5 x10^{11} vg Follow-up • 3 to 24 months	Safety • No major immune-related adverse events Visual function outcomes • 3 low, 3 medium, and 1 high dose patient improved in VA • All patients improved in GVF and subjective visual function in dim lighting • All 11 patients tested improved in PLR • 5 of 7 patients tested improved in FST • 4 of 11 patients tested improved in mobility testing
Jacobson et al 2012[43]	Phase I/2 dose-escalation	Patient population • 15 patients • Unilateral, worse eye treated except for 1 patient who had fellow eye treated because of keratoconus in worse-seeing eye • 11 to 30 years old Doses • 5.96 x10^{10} vg • 7.95 x10^{10} vg • 8.94 x10^{10} vg • 11.92 x10^{10} vg • 17.88 x10^{10} vg Follow-up • 1 to 36 months	Safety • No major immune-related adverse events • One patient developed a retinal detachment • One patient developed choroidal effusion • 3 of 5 patients with fovea-involving injections developed foveal thinning • One patient with non-fovea-involving injection developed foveal thinning Visual function outcomes • 11 of 12 patients tested improved in dark-adapted static VF • All except 1 patient improved in FST • Mean PLR showed a clinically significant improvement • 3 of 6 patients tested improved in mobility testing

(*Continued*)

Table 1 (Continued).

Study	Trial Phase	Methods	Main Results
Bainbridge et al 2015[45]	Phase 1/2 dose-escalation	Patient population • 12 patients • Unilateral, worse eye treated • 6 to 23 years old Doses • 1 x10^{11} vg • 1 x10^{12} vg Follow-up • 3 years	Safety • No major systemic immune-related adverse events • 3 patients in the high dose group developed intraocular inflammation • 6 of 10 patients with fovea-involving injections had retinal thinning Visual function outcomes • One patient improved in VA in both the treated and untreated eyes while 3 patients showed a decline • 6 patients improved in dark-adapted perimetry • 5 patients improved in microperimetry • 3 patients improved in low light navigation
Weleber et al 2016[46]	Phase 1/2 dose-escalation	Patient population • 12 patients • Unilateral, worse eye treated • 6 to 39 years old Doses • 1.8 x10^{11} vg • 6 x10^{11} vg Follow-up • 2 years	Safety • No major immune-related adverse events Visual function outcomes • 5 patients improved in VA in treated eye while 1 lost VA in both treated and untreated eyes • 11 patients improved while 5 patients had decrease in either total or central 30 degrees static VF testing at 1 or more visits • 4 patients improved while 2 patients showed decrease in kinetic VF testing • One of 4 patients with baseline minimally recordable ERGs showed a small improvement in photopic and scotopic responses • 11 patients reported improvements while 1 patient reported worsening in the NEI-VFQ-25
Bennett et al 2016[49]	Phase 1 fellow eye sequential treatment	Patient population • 11 patients treated • The fellow eye which was previously not elected for treatment was subsequently treated • 11 to 46 years old Dose • 1.5 x10^{11} vg Follow-up • 3 years	Safety • No major immune-related adverse events • one patient developed bacterial endophthalmitis • One patient developed retinal thinning Visual function outcomes • One patient improved while 1 patient had worsening in VA • 4 patients improved in GVF corresponding to injection site while 1 patient had decreased overall GVF but preserved GVF at injection site • 8 of 10 patients tested had improved FST • All patients improved in pupillary response • 8 of 10 patients tested improved in mobility testing • 8 of 8 patients tested showed increased cortical activation on fMRI
Russell et al 2017[51]	Phase 3	Patient population • 29 patients • Worse-seeing eye treated first followed by fellow eye 6–18 days later • 4 to 44 years old Dose • 1.5 x10^{11} vg Follow-up • 1 year	Safety • No major immune-related adverse events • 3 patients developed transient ocular inflammation • 2 patients developed retinal tears • One patient developed a macular hole Visual function outcomes • Bilateral mean MLMT change score was greater in treated versus control (1.8 vs 0.2), meeting primary study endpoint • Mean FST improvement >2 log units in treated versus no change in controls • Mean change in sum total degrees for GVF was +302.1 in treated versus −76.7 in controls • Mean macular threshold on HVF improved by 7.7 dB in treated versus 0.2 dB in control patients

Abbreviations: dB, decibels; ERG, electroretinogram; FST, full-field sensitivity threshold testing; fMRI, functional magnetic resonance imaging; GVF, Goldmann visual fields; MLMT, multi-luminance mobility testing; NEI-VFQ-25, National Eye Institute visual function questionnaire 25; PLR, pupillary light reflex; VA, visual acuity, VF, visual field; vg, vector genomes.

MLMT involved navigating an obstacle course at 7 different light levels resembling real-world environments. The lowest light level was 1 lux which equates to a moonless night, and the highest was 400 lux which mimics the illumination of a well-lit office space. A score was given for passing the course with higher points being awarded for passing at lower light levels. The highest possible score was 6 points for successfully navigating the course at 1 lux, and the point values decreased at each increasing light level with 0 points being awarded for passing at 400 lux. Accuracy and time scores were combined to determine a passing or failing score. The patients were allowed to attempt the course at progressively lower light levels until either failure occurred at any light level or success occurred at 1 lux. A MLMT change score was defined as the difference in the MLMT score at 1 year compared to the baseline MLMT score.[50]

The initial MLMT validation studies were done by comparing normal controls to patients with various IRDs including RP, LCA, choroideremia, Stargardt disease, and Usher syndrome. Children as young as 4 years old were able to complete the obstacle course. Passing or failing grades were given after review of the MLMT course videos by multiple masked graders and showed an inter-grader reliability of 97.9%. Based on the time and accuracy scores, MLMT had an excellent ability to distinguish between normal vision patients and patients with visual impairment from IRDs. MLMT also showed the ability to detect disease progression over 1 year in the IRD patient populations. This was especially apparent in the LCA and RP cohorts. Due to these results, the MLMT score was used as the primary outcome measure in the *RPE65* gene replacement therapy phase 3 human trials.[50]

Landmark Phase III Human Study

The phase 3 clinical trial enrolled 31 patients between the ages of 4 to 44 years old and randomized them 2:1 to intervention versus control. Individuals with a confirmed biallelic *RPE65* gene mutation could be included if they were gene therapy- or oral retinoid therapy-naïve, had best-corrected VA (BCVA) ≤20/60 (Snellen), had <20 degrees of VF in any meridian, and could perform the MLMT protocol using both eyes. Viable retinal cells had to be present as determined by a combination of fundus photography, clinical examination, and OCT retinal thickness map >100 microns in the posterior pole. The primary outcome measured was the change in bilateral MLMT performance at 1 year compared to baseline. Video recordings of the MLMT were graded at an independent reading center by two separate well-trained masked graders. The human *RPE65* cDNA carried within an AAV2 viral vector, named voretigene neparvovec-rzyl (VN), included a modified Kozak sequence at the translation site and hybrid chicken beta-actin promoter linked to a cytomegalovirus enhancer to optimize transgenic gene expression within RPE cells.[51] A dose of 1.5×10^{11} vg was chosen due to success and safety seen in prior studies.[40,48,49] Patients were temporarily immunosuppressed using pre-operative systemic prednisone 3 days prior to subretinal injections. Both eyes were treated with a 1 to 2 week delay between first and fellow eye treatments. One patient in each group left after consent but before intervention, resulting in a final intention-to-treat analysis of 20 patients in the treatment group and 9 in the control group. The control group was permitted to crossover to the treatment group at the one-year timepoint.[51]

One-year results met the primary efficacy endpoint. The mean bilateral MLMT score change showed a statistically significant improvement by 1.8 light levels in the VN group versus only 0.2 light levels in the controls ($P = 0.0013$). Improvements were seen as soon as 30 days post-treatment and were stable at 1 year. Mean unilateral MLMT score change was very similar to the bilateral mean MLMT score change. Thirteen out of 20 patients (65%) in the VN group were able to pass the MLMT at the lowest luminance level (1 lux) at 1 year as opposed to none in the control group. Mean FST improved by over 2 log units in the VN group compared to none in the control group ($P = 0.0004$). The 18 out of 20 patients who improved in MLMT change scores were also the ones who improved in FST. Mean sum total degrees of GVF nearly doubled in the treatment arm while it decreased in the control arm. There was a trend towards improved VA, but this was not clinically significant ($P = 0.17$). At 1 year, there were no major AAV2 vector-related adverse events or systemic side effects. Most common ocular side effects appeared to be surgery-related and included elevated intraocular pressure (20%), cataracts (15%), ocular inflammation (10%), retinal tears (10%), macular holes or degeneration (5%), and epiretinal membranes (5%). Of note, one treated patient experienced a large decrease in post-operative VA, and was also the only patient who did not show improvement in MLMT performance (Table 1). The overall positive results seen in the phase 3 human trial led to the FDA approval of VN for the treatment of *RPE65*-associated IRDs.[51,52]

Long-Term Phase 1 and 3 Follow-Up

Maguire et al reported long-term follow-up data on 20 patients from the original intervention (OI) group and

9 in the control-crossover (CC) group from the phase 3 trial along with 8 bilaterally-treated patients from a phase 1 contralateral eye retreatment study.[53] Updated MLMT change scores were reported at 1 year for the CC group, 2 years for the OI group, and 4 years for the phase 1 patients with gains of 2.1, 1.9, and 2.4, respectively. Improvements in FST were again highly correlated to gains in MLMT change scores. Results were stable at 1 to 4 years with 89% of patients who improved on FST being able to successfully pass the MLMT at the lowest light level (1 lux). VA again showed no statistically significant improvements at 1 to 4 years, although no significant decline was seen either. VF testing was too variable in the phase 1 patient group to allow for statistical comparison, likely due to the poorer baseline VA. GVF for the phase 3 patients were reported using the III4e stimuli. The CC group showed a 49% increase at 1 year compared to a 16% loss in the prior year before crossing over. The OI group had a 99% increase at 2 years compared to baseline. No gene therapy-related immunologic systemic adverse effects were observed. All ocular side effects were attributed to the expected risks from vitrectomy surgery.[53] Subsequent follow-up at 3 years for the CC cohort and 4 years for the OI cohort showed mean MLMT change scores of 2.4 and 1.7 light levels, respectively. Within the OI cohort, 4 out of 20 patients had a decrease of 1 lux which was still above their baselines; one patient had a gain of 1 lux. The authors suggested that amblyopia is unlikely to be a major deterrent to gene therapy, but acknowledged that there may be other ongoing degenerative processes that may limit outcomes after gene therapy.[54] Ongoing long-term follow-up studies will provide additional information in the years to come.[55]

Post-Approval Use of Voretigene Neparvovec-rzyl

Approval and Launch

Positive results from the phase 3 human trial led to the FDA approval of voretigene neparvovec-rzyl (Luxturna®) on December 19, 2017 for the treatment of patients with confirmed biallelic *RPE65* mutation-associated retinal dystrophy, marking the first-ever FDA-approved gene therapy. Included in the indication is the condition that the patient must have viable retinal cells remaining, as determined by the treating physician.[52] On November 23, 2018, approval was granted for use in all 28 member states of the European Union as well as Iceland, Liechtenstein, and Norway.[5] Spark Therapeutics decided to roll out the product at a limited number of Ocular Gene Therapy Treatment Centers around the country. These sites were selected by the company based on specialized scientific or clinical experience with VN, the ability to properly store and prepare VN, the ability to coordinate and manage patient logistics with the Spark Therapeutics team, and formal training of pharmacy and surgical specialists on proper VN handling. Currently, there are ten sites in the United States which have been approved: Bascom Palmer Eye Institute, Baylor College of Medicine, Casey Eye Center, Children's Hospital of Philadelphia, Cincinnati Children's Hospital, Kellogg Eye Center, Massachusetts Eye and Ear, Scheie Eye Institute, University of Iowa Hospitals and Clinics, and The Vision Center at Children's Hospital Los Angeles.[56]

Dosage and Safety

The medication supplied to treatment centers includes a 2-mL vial containing 0.5 mL of the medication at a concentration of 5 $x10^{12}$ vg/mL and two 2-mL vials of diluent. The initial concentration needs to be diluted 1:10 prior to subretinal administration to create a final recommended concentration of 1.5 $x10^{11}$ vg in 0.3 mL of solution. Three days prior to surgery, it is recommended that patients start a 7-day course of systemic corticosteroids equivalent to 1 mg/kg/day of oral prednisone (max 40 mg/day) followed by a taper over 10 days. It is recommended that the contralateral eye be treated within a close time interval, but with a minimum delay of 6 days between eyes. Pediatric use for children under 1 year of age is not recommended because of the active retinal cell proliferation occurring during this age which can result in possible dilution and loss of efficacy.[6]

Results from Phase 1 and 3 studies from 41 patients (81 eyes) showed no significant immune-related or systemic adverse events related to initial and repeat exposure to VN through 4 years. Transient AAV2 vector antigen and Ab may rarely be found in tears or blood without evidence of immunologic sequalae. Most ocular side effects were minor and consistent with what is expected after vitrectomy surgery including cataracts (19%), conjunctival hyperemia (11%), increased intraocular pressure (10%), and retinal tears (5%). More serious ocular events were rare and included foveal thinning (2%), endophthalmitis (1%), and retinal detachment (1%). Most reports of foveal thinning suggested that this was a result of the surgically-induced retinal detachment rather than medication-induced retinal toxicity or progressive retinal degeneration. No

clinical safety data exists for females who are pregnant or lactating or for the geriatric population.[6,49,51,53]

Preparation and Surgical Technique

VN must be prepared and diluted within 4 hours of surgery. To prepare the medication, 0.3 mL of concentrated VN and 2.7 mL of diluent are injected into a 10-mL glass vial and inverted gently a few times to allow for even mixing. Then, two 1-mL syringes are used to draw out 2 aliquots of 0.8 mL of the diluted VN mixture (one syringe serves as a backup) and set aside at room temperature for surgery. At the time of surgery, the patient is prepped and draped in usual aseptic manner; most patients are treated under general anesthesia, especially if they are of childhood age. One of the 1-mL syringes containing the prepared VN mixture is connected to polyvinyl chloride extension tubing and attached to a 41-gauge subretinal injection cannula (Figure 3). The extension tubing should not exceed a length of 15.2 cm and an inner diameter of 1.4 mm to avoid excessive "dead space" volume during priming.[6] The syringe is primed by depressing the plunger until a few droplets of the medication is seen at the tip of the injection cannula. Some surgeons will choose to express the solution until 0.3 mL is left in the syringe to ensure that the intended delivery volume is preset while others elect to self-gauge the 0.3 mL volume at time of injection in case extra volume is needed.

A standard 3-port pars plana vitrectomy is performed along with the induction of a posterior hyaloid detachment. Some surgeons elect to use triamcinolone acetonide for hyaloidal staining while others do not as its interaction with VN is unknown.[57] Once the vitreous has been removed, the injection site is inspected. It is recommended to select an area near the superior arcade that is at least 2 mm away from the foveal center and avoids retinal vessels or obvious pathology. Areas of dense atrophy or pigmentary changes should be avoided as these areas may be excessively adherent to the underlying RPE rendering subretinal bleb creation and propagation more difficult and traumatic.[6,58] The tip of the injection cannula is trimmed, and sometimes beveled, to prevent kinking during cannula insertion and to facilitate entrance into the subretinal space.[59] Once the subretinal cannula is in contact with the retina, slow and steady injection of drug initiates bleb creation (Figure 4). Of note, some surgeons will first create a pre-bleb with balanced salt solution or air, but how this affects the concentration of gene therapy is not well-understood.[57] Microscope-integrated OCT may be used to confirm that the bleb has been created within the correct tissue plane (Figure 5). The remaining VN solution is injected into the bleb until the entire 0.3 mL is delivered.

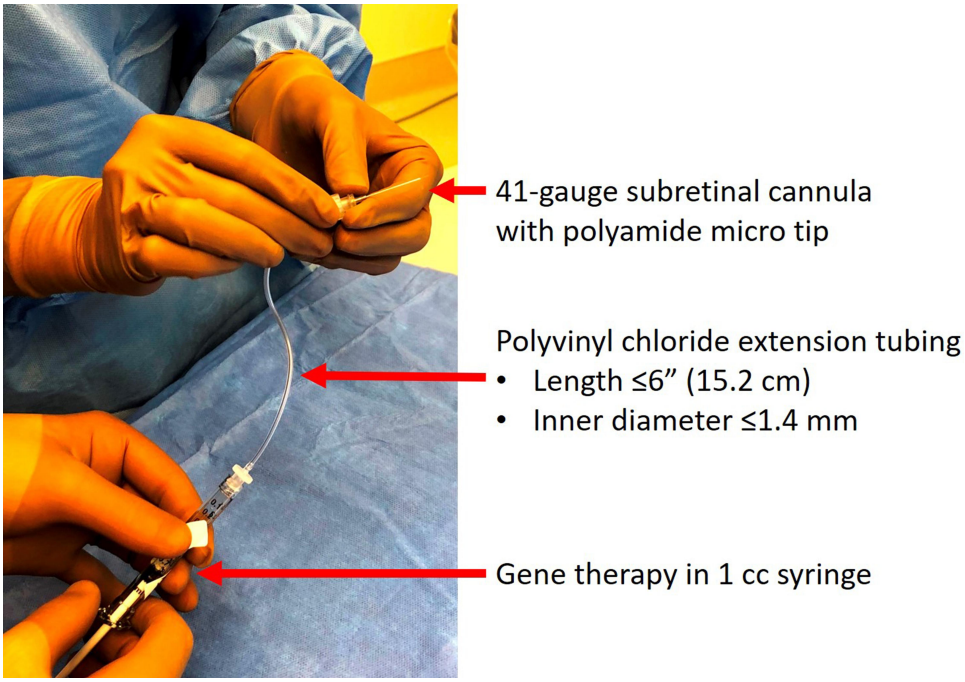

Figure 3 Image illustrating the set-up of the injection apparatus. A 1-mL syringe containing the prepared voretigene neparvovec is connected to extension tubing and a 41-gauge subretinal injection cannula.

Figure 4 Intraoperative photograph illustrating a fovea-involving subretinal bleb created by injecting 0.3 mL of voretigene neparvovec.

A foot pedal-assisted injection method has also been described using infusion pressures around 12–16 psi; this eliminates the need for a surgical assistant.[58,60] Care should be used to avoid over-stretching the bleb, especially if the fovea is involved, as an iatrogenic full-thickness macular hole can be created. Another injection site may be used if over-stretching of the first bleb is of concern.[59] To stabilize bleb localization and possibly mitigate reflux of viral vector into the vitreous cavity, an air-fluid exchange is performed with care to avoid aspirating directly over the injection site. Instruments are removed and all sclerotomies are sutured. Post-operatively, patients are advised to remain supine for the next 24 hours so that the medication can diffuse over the macula. Usual post-vitrectomy precautions, eye drops, and follow-up visits are recommended.[57]

Cost-Effectiveness

The voretigene neparvovec-rzyl drug itself costs $425,000 per eye. When accounting for facility and surgical fees, the total cost is even higher.[61] Some have raised concerns regarding the cost of this therapy relative to the actual benefits received.[62] Since the 1990s, the incremental cost-effectiveness ratio (ICER) has been established as a metric for evaluating cost-effectiveness of medical therapies. ICER is calculated by dividing the cost of therapy by the quality-adjusted life years (QALY). QALY is defined as the life expectancy multiplied by the quality of life on a scale of 0 to 1 (1 equates to perfect health while 0 equates to death). ICER is reported as cost per QALY. An ICER range of $50,000 to $150,000 United States dollars (USD), which equates to approximately 45,000 to 137,000 euros, has been generally considered as the threshold range for cost-efficiency such that anything at or below this range is considered to be cost-effective.[63,64]

Johnson et al used VA and VF data from a retrospective natural history study of *RPE65*-associated IRD patients and compared their progression to that of patients from the gene therapy trials. Direct medical costs from visual impairment were estimated based on a neovascular age-related macular degeneration (nAMD) study population of 200 patients with an adjustment for inflation. Indirect costs were approximated using national surveys evaluating productivity loss, caregiver burden, and

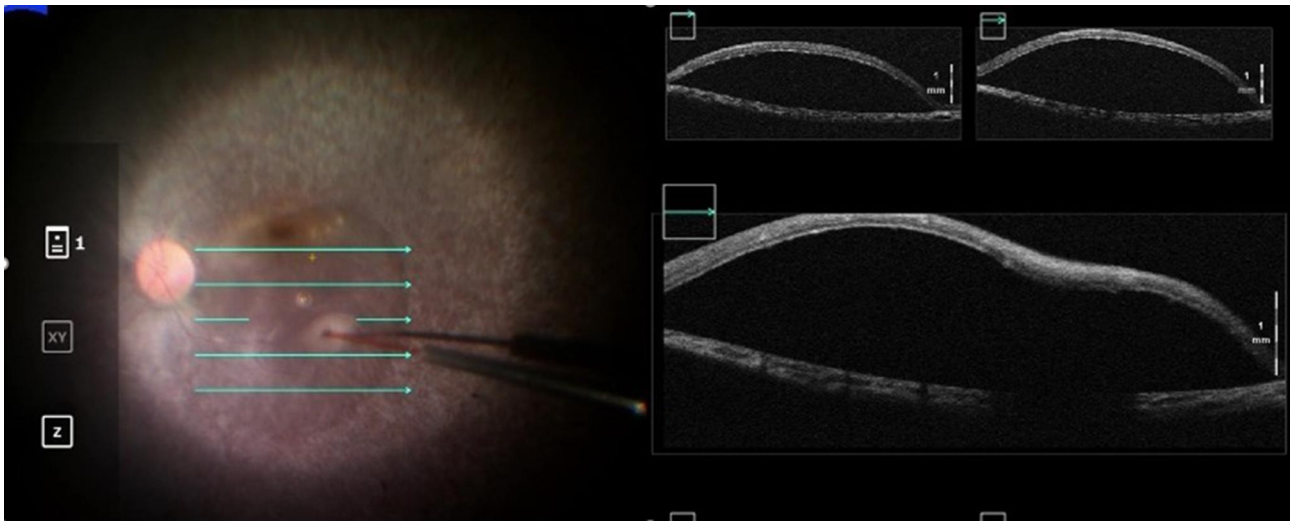

Figure 5 Intraoperative optical coherence tomography imaging can be used to guide the formation of a subretinal bleb of voretigene neparvovec and confirm its proper localization in the subretinal space.

government program costs for patients with IRD. A standard 3% annual discount for costs and benefits was included. Assuming gene therapy benefits were completely lost after year 3, the study estimated an ICER of $380,185 and $237,140 per QALY excluding and including the burdens of indirect costs, respectively. This exceeds the standard range accepted as cost-effective. However, the study calculated that if at least 8.8% (including indirect costs) or 43.3% (excluding indirect costs) of the long-term treatment effects persisted after year 3, then VN therapy would fall below the $150,000 per QALY threshold to be considered cost-effective.[61] Another group using slightly different assumptions and estimates also found VN to be cost-ineffective. Their study assumed a 10-year duration of treatment effect followed by a 10-year waning period. The resulting ICER value accounting for average total lifetime direct medical costs without gene therapy was $643,813 per QALY and $480,130 per QALY when nonmedical and indirect costs were also accounted for.[65] Unlike the prior two studies, a United Kingdom study assumed a 40-year duration of treatment effect, and did find VN to be cost-effective. Costs calculations include VN acquisition price, administration, testing, monitoring, and adverse events. The study found a final ICER of 95,072 euros per QALY.[66]

A major limitation in all these studies is the use of VA and VF as the primary outcomes representing benefit from therapy. These two markers have been found to be extremely variable and are unlikely to be accurate reflections of the benefits gained from gene therapy in *RPE65*-associated IRDs. MLMT or FST may be better metrics for visual function gains, but have not been used because no cost-association data exists for either. Other limitations include the unknown true durability of VN therapy, variability in assumptions incorporated into the costs, and the visual function results being extrapolated from small sample sizes due to the rarity of the disease.

Spark-Sponsored Patient Assistance

Spark Therapeutics has enacted several strategies to improve patient access to voretigene neparvovec treatment in light of prohibitory high costs. One includes a unique outcomes-based rebate model which shares the cost responsibilities with the payer if outcomes fail to meet predetermined thresholds. These thresholds will be based on short-term (30–90 days) and long-term (30 months) FST testing scores. The second involves bypassing the typical billing model known as "buy and bill" in which the treating facility first purchases a drug and later initiates a bill to the payer for reimbursement. Instead, Spark Therapeutics offers a different contracting model where the company enters into a direct agreement with commercial payers for the purchase of VN which may cap patient out-of-pocket expenses at an in-network limit. The third involves a proposal to be reviewed by the Centers for Medicare and Medicaid Services for a long-term payment model that would allow the costs of therapy to be paid over several years rather than as a single lumpsum payment upfront. This also increases the possibility of receiving company rebates linked to clinical outcomes. If this proposal is rejected, the company will propose that its distributors be permitted to independently make alternative payment options available to the payer which may include installments or financing options. The company has also developed a team known as the Spark Therapeutic Generation Patient Services which assists commercially insured patients in navigating the insurance process. This includes support for travel and lodging costs related to treatment as well as other out-of-pocket treatment-related costs.[67–69]

Conclusion

Over the last decade, gene therapy research has made significant progress, and new mutations are being discovered at a rapid pace.[70] The success of VN in human clinical trials has paved the way for investigational studies targeting other genetic mutations associated with a variety of IRDs. Currently, there are dozens of ongoing gene therapy trials at both preclinical and clinical stages for diseases such as achromatopsia, X-linked retinoschisis, X-linked retinitis pigmentosa, choroideremia, and even non-IRDs such as neovascular age-related macular degeneration and diabetic macular edema. In addition to addressing the mutations themselves, scientists are also evaluating factors that contribute to the pathogenesis of IRDs. For example, oxidative stress may induce alternative gene expression pathways, contributing to progression of certain IRDs.[71,72] As our knowledge about gene therapy and IRDs expands, so does the likelihood of having more therapeutic options to treat once-untreatable blinding diseases.

Disclosure

Dr Rehan M Hussain reports personal fees from Alimera Sciences, outside the submitted work. Dr Christina Y Weng reports personal fees from Alcon, Inc., Alimera

Sciences, Inc., Allergan/AbbVie, Dutch Ophthalmic Research Center, Novartis, Regeneron, and REGENXBIO, outside the submitted work. The authors report no other conflicts of interest in this work.

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