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Voretigene neparvovec-rzyl for treatment of *RPE65*-mediated inherited retinal diseases: a model for ocular gene therapy development

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ABSTRACT

Introduction: Over a decade of research and development culminated in the 2017 United States (US) Food and Drug Administration (FDA) approval of voretigene neparvovec-rzyl (VN) for *RPE65* mutation-associated inherited retinal disease (IRD), the first approved gene therapy for a hereditary genetic disease in the US, and the first and only pharmacologic treatment for an IRD.

Areas covered: VN serves as a model for ocular gene therapy development, while *RPE65* mutation-associated IRD serves as an example of a well-suited candidate disorder. This review also discusses development considerations for viral vector gene augmentation, and, studies that led to VN's FDA approval. Subretinal injection of VN resulted in improved performance on the novel multi-luminance mobility test (MLMT), light sensitivity, and visual fields in patients with *RPE65* mutation-associated IRD, which predominantly impairs rod function. Additionally, the dosage, administration technique, pharmacokinetics, and safety data of VN are reviewed.

Expert Opinion: As a model for development, special challenges associated with the introduction of this first ocular gene therapy include limited genetic testing in clinical practice, novel surgical complexity of ocular gene therapy administration, new functional vision endpoints, as well as unique development, launch, and reimbursement considerations associated with orphan therapies and one-time gene therapies.

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Gene therapy; adeno-associated virus; inherited retinal disease; Leber's congenital amaurosis; retinitis pigmentosa; voretigene neparvovec-rzyl; luxturna; *RPE65*

1. Introduction

Inherited retinal diseases (IRDS), rare heterogeneous disorders with causative mutations in over 260 genes, generally lead to progressive retinal degeneration and severe visual impairment. Historically untreatable, the most common IRDs include Stargardt disease, retinitis pigmentosa (RP), Usher syndrome, Leber's congential amaurosis (LCA), choroideremia, achromatopsia, and X-linked retinoschisis (XLRS) [1]. In 2017, the United States (US) Food and Drug Administration (FDA) approved voretigene neparvovec-rzyl (VN, Luxturna, Spark Therapeutics, Philadelphia, PA, USA) to treat *RPE65* mutation-associated IRDs, rendering it the first approved gene therapy for a hereditary genetic disease in the US [2]. In November 2018, the European Commission approved its use in all 28 member states of the European Union, as well as Iceland, Liechtenstein, and Norway [3].

VN, a genetically modified non-replicating adeno-associated virus (AAV) serotype 2, facilitates the expression of the human *RPE65* transgene. It has been approved for the one-time treatment of confirmed biallelic *RPE65* mutation-associated IRDs with viable retinal cells as determined by treating physicians [4]. The efficacy of VN was established on the basis of multi-luminance mobility testing (MLMT) score change from Baseline to Year 1 [4]. The MLMT measures changes in functional vision, the ability to conduct visually dependent activities of daily living, as assessed by the ability of a subject to navigate a course accurately and at a reasonable pace at different levels of environmental illumination.

Autosomal recessive *RPE65* mutation-associated IRD was a well-suited candidate disorder for the development of ocular gene therapy, and VN serves as a model for the development of other gene therapies to treat IRDs. Consequently, this review will discuss ocular immune privilege, *RPE65*-associated IRD, gene augmentation using viral vectors with an emphasis on AAV2, and history of VN development. It will also cover challenges associated with the introduction of this first ocular gene therapy, including novel surgical complexity of ocular gene therapy administration, limited genetic testing in clinical practice, new functional endpoints such as the MLMT, as well as unique development, launch, and reimbursement considerations associated with orphan therapies and one-time gene therapies.

2. Ocular immune privilege, a key catalyst for early ocular gene therapy development

The eye provides an excellent model for investigating gene therapy given the large number of monogenic disorders (270

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Article highlights

- The 2017 United States (US) Food and Drug Administration (FDA) approval of voretigene neparvovec-rzyl (VN, Luxturna, Spark Therapeutics), the first US gene therapy for a genetic disease, marked a new cycle of innovation in ophthalmic therapies. VN serves as a model for the development of gene therapies to treat other IRDs. However, autosomal recessive *RPE65* mutation-associated inherited retinal disease (IRD) may have been particularly well-suited for gene therapy development, as it involves a key enzymatic defect, and augmentation of even a small amount of enzymatic activity can sometimes restore phenotype, while it does not cause irreversible degeneration until later in its course; development efforts also benefited from a large animal model.
- Voretigene neparvovec-rzyl is a non-replicating adeno-associated virus (AAV) serotype 2, which has been genetically modified to express the human *RPE65* transgene; it has been approved for the one-time treatment of confirmed biallelic *RPE65* mutation-associated IRD with viable retinal cells as determined by treating physicians. VN restores the visual cycle via functional retinoid isomerohydrolase, a 65-kD protein expressed in the RPE.
- The efficacy of VN was established on the basis of multi-luminance mobility testing (MLMT) score change from Baseline to Year 1. While practitioners have historically relied on visual acuity (VA) as a primary endpoint, many IRDs primarily affect rods and peripheral vision with late effects on VA. Tests of functional vision, the ability to conduct visually dependent activities of daily living, are important because they correlate with quality of life. The MLMT was designed to measure changes in functional vision, as assessed by the ability of a subject to navigate a mobility course accurately and at a reasonable pace in different levels of environmental illumination. MLMT and other tests of functional vision will become increasingly important as additional gene therapies for IRDs undergo clinical study and potential commercialization.
- Patients in the phase 3 trial of VN have shown durability of response to 4 years. Mean changes in MLMT at Year 1 were maintained at Year 4 for the original intervention group.
- VN administration to the RPE involves pars plana vitrectomy (PPV) followed by transretinal injection into the subretinal space. Precise subretinal administration of gene therapy via PPV is an evolving procedure, and its novel surgical complexity represented a challenge in the introduction of VN. For rare genetic disorders, ocular gene therapy treatment centers consolidate care, to facilitate safety and treatment optimization, as consistent patient and procedural volume in rare disease drives a virtuous cycle of expertise and positive outcomes.
- Gene therapy requires a genetic diagnosis, yet there has been limited clinical use of genetic testing in retina practices, representing another hurdle in the launch of VN. Educational efforts to address the complexity of genetic testing results must be continued for the successful adoption of precision medicine.
- Orphan therapy development, launch, and reimbursement considerations represented another group of challenges in the launch of VN, as ophthalmologists generally have not been exposed to treatments approved for orphan diseases. The launch of VN spurred the development of multiple innovative solutions.

This box summarizes key points contained in the article.

Box 1. Drug summary.

Name of therapy:	Voretigene neparvovec-rzyl (Luxturna, Spark Therapeutics, Philadelphia, PA, USA)
Indication:	Confirmed biallelic *RPE65* mutation-associated retinal dystrophy, with viable retinal cells as determined by the treating physician(s); not recommended for patients younger than 12 months of age
Dates of approval:	December, 2017: United States Food and Drug Administration; November 2018: European Commission (all 28 member states of the European Union, as well as Iceland, Liechtenstein, and Norway)
Dosage and route of administration:	1.5×10^{11} vector genomes, administered by subretinal injection in a total volume of 0.3 mL, to each eye on separate days within a close interval, but no fewer than 6 days apart; systemic oral corticosteroids are recommended, equivalent to prednisone at 1 mg/kg/day (maximum of 40 mg/day) for a total of 7 days (starting 3 days before administration of voretigene neparvovec-rzyl to each eye), followed by a tapering dose during the next 10 days
Mechanism of action:	Delivers to retinal cells a normal copy of the gene encoding the human retinal pigment epithelial 65 kDa protein (RPE65), in patients with reduced or absent levels of biologically active RPE65
Pivotal trial:	Russell S, Bennett J, Wellman JA, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. Lancet. 2017;390(10097):849–860.

+), accessibility to target cell delivery, the noninvasive ability to monitor for disease progression or therapeutic response, as well as relative immune-privilege which limits inflammatory response [5]. Although viral vector capsid antigens can invoke immunogenicity, ocular immune-privilege limits an immune response to subretinally injected vector, while the tight blood-ocular barrier limits the systemic dissemination of the introduced genetic material [6].

Ocular immune privilege was originally studied in the context of corneal transplantation. This immune privilege may result from immune ignorance (absence of lymphatic draining, absence of MHC class-II expressing antigens presenting cells that normally activate alloreactive T effector cells, and angiostasis), anterior chamber–associated immune deviation (ACAID, peripheral tolerance of eye-derived antigens, which is also noted in the vitreous cavity and subretinal space (SRS), leading to suppressor T cells that downregulate delayed-type cellular immunity), and an intraocular immunosuppressive microenvironment (due to soluble and cell-surface immune-modulatory factors) [7].

In the SRS, murine studies have demonstrated immune deviation for histoincompatible tumor cells and soluble protein antigens by actively suppressing antigen-specific delayed-type hypersensitivity [8]. When the outer blood-retinal barrier is experimentally disrupted, acute loss of immune privilege in the SRS and the vitreous cavity does not cause loss of privilege in the anterior chamber (AC); however elimination of immune privilege in the AC eliminates the capacity of the SRS and the vitreous cavity to support immune deviation to antigens injected locally. A subsequent section, on ocular gene therapy administration, further discusses immune response with respect to subretinal and intravitreal viral vector gene therapy administration.

For gene therapy, one issue of interest involves the possible increased risk of an ocular immune response, due to previously introduced AAV2 vector in a prior-treated fellow eye. Inflammation directed at the viral vector would not only limit transfection efficacy and protein production, but also damage sensitive photoreceptors and retinal pigment epithelium (RPE), already compromised from the underlying retinal disorder. Nevertheless, no clinically meaningful deleterious

immune response was noted in the second-treated eye during the phase 1 and phase 3 VN studies, which included an immunomodulatory perioperative course of oral corticosteroids [9,10].

3. *RPE65* mutation-associated retinal dystrophy, a well-suited candidate disorder for ocular gene therapy development

Autosomal recessive *RPE65* mutation-associated IRD was well-suited for ocular gene therapy development, as it involves a key visual cycle enzyme (and augmentation of even a small amount of enzymatic activity can sometimes restore phenotype), affects the RPE (which is efficiently transduced by AAV2, in an imune privileged space, with each RPE cell subserving numerous photoreceptors to potentially yield a multiplicative treatment effect), does not cause irreversible degeneration until later in its course, and benefitted from a large animal model. It has historically been known as retinitis pigmentosa 20 (RP20) and Leber congenital amaurosis (LCA), type 2. It is a rare visually devastating disease, with an estimated prevalence of one in 50,000–100,000, caused by a variety of genetic mutations in the *RPE65* gene [11]. Classically, LCA presents with nyctalopia, nystagmus, visual field (VF) constriction and severely decreased visual acuity (VA) in early infancy [12]. However, a retrospective natural history study of 70 patients with confirmed *RPE65* mutation-associated IRD demonstrated its heterogenous nature, with a spectrum of phenotypes, including a variety of clinical diagnoses and findings, as well as variable age of onset, rate of progression, and severity [13]. At the first reported visit, clinical diagnoses included LCA in 47%, RP in 8%, tapetal retinal dystrophy in 5%, severe early childhood–onset retinal dystrophy (SECORD) in 5%, and early-onset severe retinal dystrophy (EOSRD) in 3%. Clinical findings were diverse and variable but included nystagmus (79%), refractive error (93%), strabismus (31%), cataract (20%), outer retinal atrophy and pigmentary abnormalities (99%), retinal vascular attenuation (91%), macular abnormalities (86%), optic disc pallor (76%), and vitreous abnormality (26%). Although the rate of change also varied, VA loss was typically severely progressive during the first decade, gradual during the second decade when it typically progressed beyond legal blindness (logMAR 1.0, Snellen equivalent 20/200), and severely progressive thereafter. Visual fields showed a similar declining trend with age, also associated with variability. Given these varying phenotypes, genetic testing is essential in diagnosing *RPE65* mutation-associated retinal dystrophy.

Pathogenic mutations in the *RPE65* gene encoding retinoid isomerohydrolase, a 65-kD protein expressed in the RPE, can disrupt its production and/or function leading to the breakdown of the visual cycle (Figure 1), critical to phototransduction [14]. When light strikes rhodopsin, the protein opsin bound to the chromophore 11-cis-retinal, it converts 11-cis-retinal to its trans isomer in the rod outer segments. This then activates the opsin and initiates a signal transduction cascade, closing a cyclic GMP-gated cation channel, and hyperpolarizing the photoreceptor cell. To complete the visual cycle, a series of RPE-based enzymes, including retinoid isomerohydrolase and Lecithin Retinol Acyltransferase (LRAT), convert 11-cis retinal back from its trans isomer [15]. Deficiency in either of these enzymes impairs the visual cycle, leading to early-onset progressive degeneration of rod photoreceptors and ultimately results in irreversible loss of cone-mediated vision. Gene therapy, with complementary DNA (cDNA) encoding deficient enzyme, can restore the visual cycle and improve visual function.

4. Development considerations for viral vector gene augmentation

4.1. Vectors for ocular gene augmentation

Although a detailed review is beyond the scope of this article, readers should be familiar with the major categories of gene therapy, including *gene augmentation* (adding a gene to a cell), *gene editing* (revising the existing genetic code), *gene inactivation* (silencing a gene, often a dominant-negative one) and *selective toxicity* (introducing 'suicide' genes, and immune sensitization as in chimeric antigen receptor, or CAR, T cells to recognize cancer cells).

Gene augmentation, the introduction of a normal copy of a gene, is the therapeutic strategy behind VN. Recessive single-gene disorders, such as biallelic *RPE65* mutation-associated IRDs, are generally the most amenable to gene therapy because the mutations causing the disease generally lead to absence or severely decreased functional protein with resulting loss-of-function of the normal gene product. In these recessive single-gene disorders, augmentation can ameliorate the lost function through delivery and expression of a normal gene. As noted previously, restoring even a small percentage of the normal gene product is sometimes sufficient to revert the phenotype, particularly when restoring enzymes, like retinoid isomerohydrolase (encoded by the *RPE65* gene). Most current or investigational gene therapies for IRDs target autosomal recessive diseases. In contrast, gain-of-function mutations found in autosomal dominant disease may be less amenable to gene augmentation because 1 allele expresses an abnormal product that must be suppressed [16]. Consequently, developing a gene therapy for some forms of autosomal dominant RP has been more challenging.

A variety of viral and non-viral gene delivery methods have been developed over the past 2 decades [5]. Choice of vector is determined by the tissue to be targeted, the cloning capacity of the vector (which determines the size of the expression cassette that can be accommodated in the genome of the virus), and safety concerns (inflammatory responses, and possibility of genotoxicity/insertional oncogenesis). AAVs and lentivirus vectors have been used in clinical trials for IRDs. Lentiviruses are RNA viruses of the retrovirus family. Commonly used lentiviral vectors derive from the human immunodeficiency virus 1 (HIV1) or the equine infectious anemia virus (EIAV) [17]. Lentivirus vectors efficiently integrate their genome into the host cell genome and do not require cell division for integration [18]. Lentiviruses, with current safety features, do not preferentially integrate into the proximity of oncogenes [19]. Lentivirus vectors have large capacity, up to 10 kb [20], and consequently have been utilized for retinal disorders involving large genes, such as *ABCA4* for

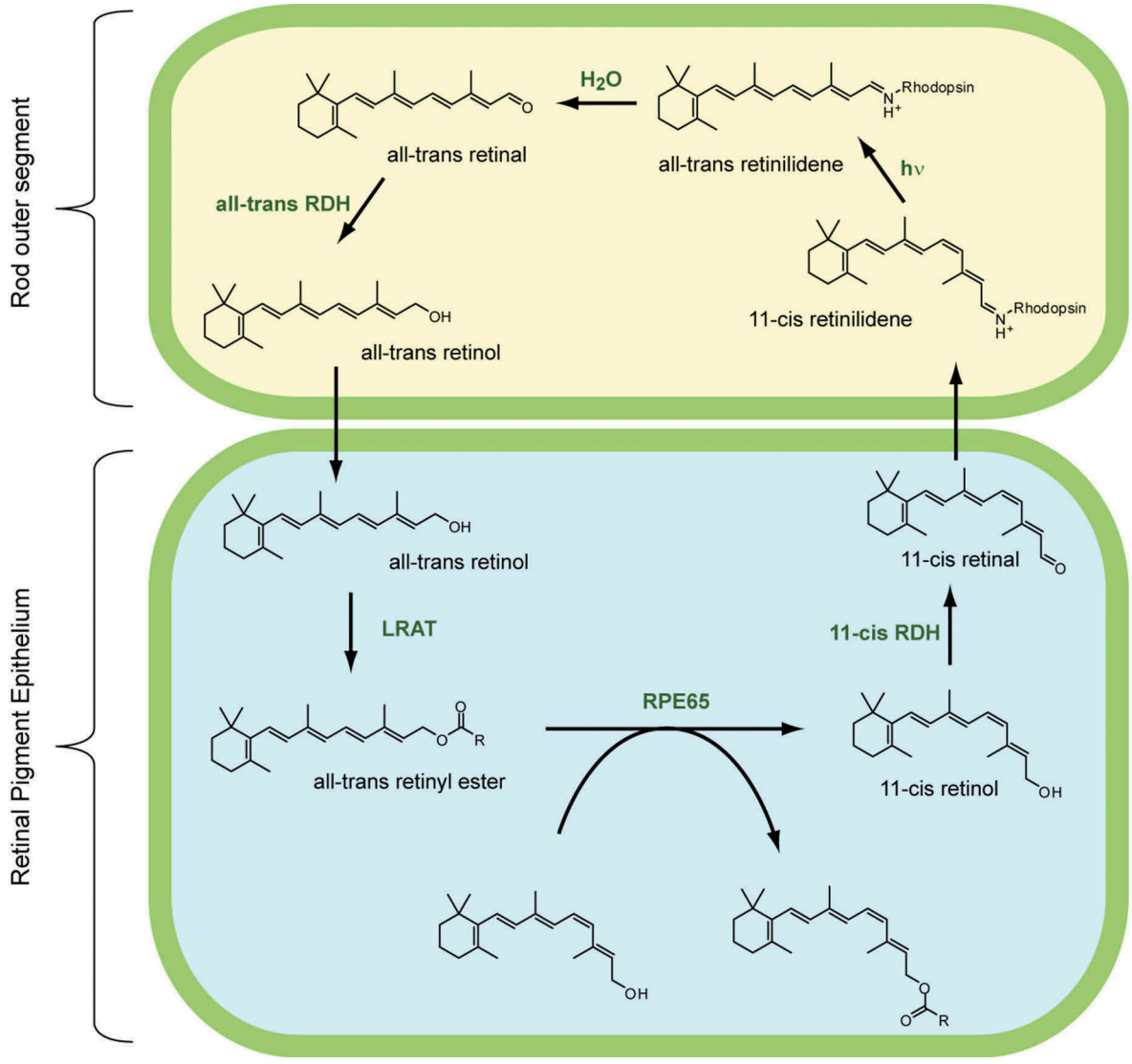

Figure 1. The visual cycle refers to an enzymatic process that takes place in the outer retina photoreceptors and RPE. Photoreceptors use 11-cis-retinal, which binds to opsins to form visual pigments such as rhodopsin or cone opsins. When light strikes rhodopsin in the rod outer segments, 11-cis-retinal is converted to its all-trans-retinal isomer. This, in turn, activates the opsin and initiates a signal transduction cascade, closing a cyclic GMP-gated cation channel, and hyperpolarizing the photoreceptor cell. In the visual cycle, the all-trans-retinal must be converted back to 11-cis-retinal via a series of steps catalyzed by enzymes, including retinol dehydrogenases (RDH), which catalyze reduction and oxidation reactions, as well as lecithin retinol acyltransferase (LRAT) and retinoid isomerohydrolase (a 65-kilodalton protein encoded by the RPE65 gene), both of which are located in the RPE. This open-access figure was obtained from https://commons.wikimedia.org/wiki/File:Visual_cycle_v2.png.

Stargardt macular dystrophy (NCT01367444, NCT01736592) and *MYO7A* for Usher Syndrome (NCT01505062).

Nevertheless, AAV is the most commonly utilized viral vector for investigational retinal gene therapy. AAVs are small (approximately 25 nm), single-stranded DNA viruses of the parvovirus family [21,22]. Multiple features render AAV an excellent vector choice for the treatment of retinal diseases, including the non-pathogenic, non-replicating, non-integrating nature, and ability to transduce non-dividing cells, as well as low immunogenicity at appropriate doses, and excellent history of safety in human trials [23]. AAV vectors do have limitations, which include having a restricted transgene capacity (4.5–5.0 kb) and the risk of being rapidly eliminated by the humoral immune response in patients who have previously been exposed to the virus [24]. However, the risk for immunogenicity with AAV vectors is low when targeting relatively immune-privileged sites such as the SRS [25].

AAV vectors transduce quiescent sites, and their genome is generally maintained as extrachromosomal monomeric and concatemeric circles. Over 100 different AAV serotypes have been described, each of them displaying enhanced tropism for a specific set of tissues depending on their capsid. AAV serotypes 2, 5, and 8 are most commonly used for retinal gene therapy, as they are capable of transducing photoreceptors and RPE [22]. In a nonhuman primate study, both AAV2 and AAV8 efficiently transduced RPE, but AAV8 was markedly better at targeting photoreceptor cells [26]. As noted previously, VN utilizes AAV2 as a vector to deliver the *RPE65* transgene, encoding retinoid isomerohydrolase, to the RPE. Figure 2 shows the AAV-2 serotype of adeno-associated virus.

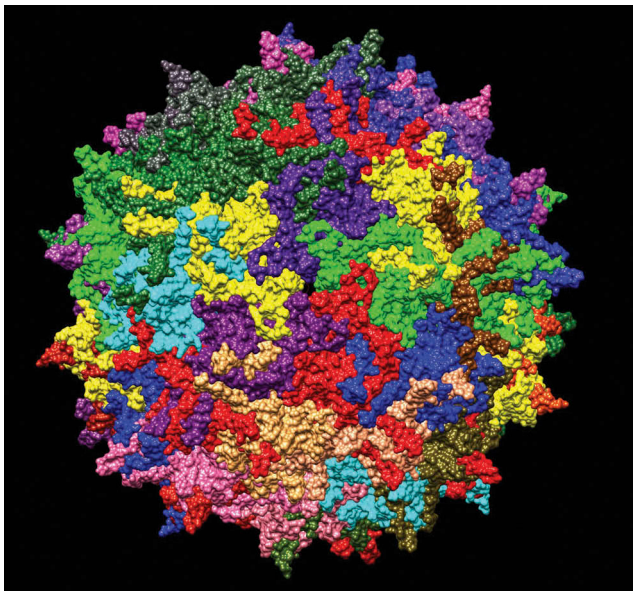

Figure 2. Surface of the AAV-2 serotype of the adeno associated virus, with one of the five fold axes centered. Derived from the 3-A crystal structure. This open-access figure was obtained from https://commons.wikimedia.org/wiki/File:Adeno-associated_virus_serotype_AAV2.jpg.

Heparan sulfate proteoglycan on the RPE surface functions as a primary receptor for AAV2 [27].

4.2. AAV gene augmentation optimization and manufacturing

Manufacturing novel gene augmentation therapies, such as VN, can be challenging. There are numerous factors involved in optimizing and manufacturing gene therapy. It is critical to note that gene augmentation therapies targeting the same gene, even with the same or similar vector capsid, can vary in multiple important ways, including codon optimization, regulatory elements, residual empty capsids, final formulation, dose optimization, surgical delivery procedure, and adjuvant immunomodulatory therapy. Within the vector, and packaged between inverted terminal repeats along with regulatory elements, the therapeutic transgene is actually cDNA, with no introns, only the coding exons. Some undergo codon optimization, which involves the introduction of synonymous mutations into recombinant genes, changing rare codons to common codons, which may improve protein translation efficiency. Promotors and enhancers partially determine where and how robustly the gene is expressed. VN utilizes a hybrid chicken β-actin promoter with a cytomegalovirus enhancer [9], regulatory elements that promote robust expression. VN also includes a modified Kozak sequence which corresponds to a translational start site in the resulting mRNA [9]. In non-dividing cells like RPE, VN's therapeutic *RPE65* transgene, encoding retinoid isomerohydrolase, is incorporated into the cell nucleus as a stable extragenic episome, and designed to be expressed constitutively.

Viral vector gene therapies are complex, requiring more complicated processing than pharmaceuticals or nonviral gene therapies because virus capsids are developed within cell lines. VN is generated by 'triple transfection' on an HEK293 cell line, which serves as a 'biofactory' to produce viral vectors. With AAV2, triple transfection involves 3 plasmid constructs carrying expression cassettes encoding the therapeutic transgene (including regulatory elements such as promoters and enhancers), the AAV (rep and cap genes), and helper virus sequences (to replicate and pack the recombinant AAV with therapeutic transgene). Multiple purification steps ensue to extract the viral capsids from the cell media. For VN, empty capsids are also substantially removed, as empty capsids potentially decrease overall transduction efficiency where RPE receptors may be limiting, and may pose a risk of immune activation. Also, for VN, a surfactant is added to prevent subsequent vector loss on product contact surfaces during storage and administration, and to enhance reproducibility of dose delivery [9].

In addition to these complex manufacturing processes, novel analytical protocols are required by regulatory authorities with numerous tests of strength, identity, purity, potency, and viral safety, including tests of transfection activity with resulting protein expression and activity. Furthermore, scaling of viral vector gene therapy manufacturing is very complex, but is already being addressed for hematologic diseases like hemophilia, for which liver-directed gene therapy requires large amounts of vector compared to retinal gene therapy. For example, Spark Therapeutics constructed its own vector manufacturing facility in 2014 and successfully scaled AAV production from a mammalian cell-adherent manufacturing process to a cell-based suspension manufacturing process in a 200-L bioreactor [28].

5. History of voretigene neparvovec-rzyl development

5.1. Preclinical studies

Preclinical animal models have facilitated the assessment of *RPE65* mutations and effect on retinoid isomerohydrolase. Gene augmentation therapy, via subretinal administration of recombinant AAV vectors containing *RPE65* cDNA, improved functional vision and electrophysiological responses in the Swedish Briard dog, a naturally occurring animal model with mutated *RPE65* [29,30]. Successful gene augmentation in preclinical murine and especially large-animal models stimulated human clinical trials evaluating the safety of subretinally injected AAV vectors containing the human *RPE65* coding sequence.

5.2. Phase 1 studies

In 2008, 3 different groups published small phase 1 trials of gene augmentation for RPE65 mutation-associated IRD [31–33]. In these studies, there were preliminary signs of efficacy and safety, with adverse events related to the surgical administration procedure. This favorable safety profile and preliminary effectiveness supported the next study by Maguire's group, a dose-escalation phase 1study of 12 patients age 8–-44 years (NCT00516477) [34]. Patients were randomized to low dose (1.5 x10^{10} vector genomes (vg) in 0.15 mL), medium dose (4.8 x 10^{10} vg in 0.15 mL), or higher dose (1.5 x 10^{11} vg in

0.3 mL) AAV2-hRPE65v2 administered subretinally following pars plana vitrectomy (PPV). The therapy was well tolerated, and all patients demonstrated sustained improvement in VA, pupillometry, nystagmus, VF, and ambulatory behavior [34]. Visual function improvements remained stable up to 3 [35] and 4 years [36]. However, Jacobson et al. and Bainbridge et al. reported less favorable long-term outcomes, although the therapies differed with respect to regulatory elements, manufacturing processes, doses, and/or administration volumes [37,38].

With encouraging results from the phase 1 trial reported by Maguire et al., Spark Therapeutics sponsored further development of this therapy. In the next phase 1 study (NCT01208389), AAV2-hRPE65v2 was administered to the contralateral eye in patients enrolled in the prior phase 1 study [10]. Since there were no dose-limiting toxicities in the first study, the highest dose of 1.5×10^{11} vg in 0.3 mL was chosen for the contralateral, previously untreated, eyes in 11 of the 12 patients. One patient was not eligible due to glaucoma in the uninjected eye. Although there was concern regarding the theoretical risk of an immune response due to the previous exposure to AAV2 in the fellow eye, most patients did not experience significant immune response.

With respect to endpoints, VA testing after gene therapy in IRDs can be complicated by amblyopia. Furthermore, predominantly rod-mediated disorders such as *RPE65* mutation-associated IRD do not primarily affect cone function and VA, decreasing its sensitivity to change in visual function. Consequently, a proprietary multi-luminance mobility test (MLMT) was developed, with feedback from the FDA, to functionally assess VA, VF, light sensitivity, and mobility. A change in score, based on the illumination level at which a subject can successfully navigate the course in under 3 min, functions as the endpoint and has been validated [39]. The MLMT is discussed in greater detail in a subsequent section. In the phase 1 study, improvements in MLMT and full-field light sensitivity testing (FST) by day 30 persisted to year 3 (MLMT $p = 0.0003$, FST $p < 0.0001$), and no significant change was seen in the previously injected eyes or VA assessments over the same time period [10].

5.3. Phase 3 study

In 2012, Spark Therapeutics sponsored a phase 3 trial, enrolling 31 patients with a mean age of 15 years; the patients were randomized 2:1 into control or receiving 1.5×10^{11} vg of VN in both eyes within 18 days [9]. One participant from each group withdrew after the consent, before intervention, leaving a modified intention-to-treat population of 20 intervention and 9 control participants. After the 12-month time point, the control subjects were eligible to cross over into the treatment group. A 1:1 randomization design would have required fewer patients to achieve the same power, but this 2:1 randomization with subsequent crossover was choosen to address potential enrollee preferences, given awareness of prior study results.. The average age of the 31 randomized subjects was 15 years (range 4–44 years), including 64% pediatric subjects (n = 20, age from 4 to 17 years) and 36% adults (n = 11). The 31 randomized subjects included 13 males and 18 females. Sixty-eight percent (68%) of the subjects were White, 16% were Asian, 10% were American Indian or Alaska Native, and 6% were Black or African-American.

In 2015, Spark Therapeutics announced positive top-line results. The trial met its primary endpoint of change in the bilateral MLMT from baseline ($p = 0.001$) with an average improvement of 1.8 light levels in the treatment group versus 0.2 light levels in the control group at 1 year [9,40]. In the VN treatment group, 13/20 (65%) subjects passed the MLMT at 1 lux (the lowest light level, demonstrating maximal possible improvement) at 1 year versus no subjects in the control group. Eleven of the 21 (52%) subjects in the VN treatment group experienced an MLMT score change of 2 or greater, while 1 of the 10 (10%) subjects in the control group experienced an MLMT score change of 2. Secondary endpoints successfully met included white light FST with ~100-fold improvement in light sensitivity in treated subjects ($p < 0.001$) and MLMT change score for the first injected eye ($p = 0.001$). In addition, the mean sum total degrees of the Goldmann visual field III4e isopter almost doubled in treated subjects compared to a decrease in the control group. Although the VA secondary endpoint did not reach statistical significance, a beneficial trend was noted among treated patients. With respect to safety, there were no serious adverse events (SAEs) or significant immune responses related to VN.

5.4. Updated results of phase 1 and phase 3 studies

Follow-up data for both the phase 1 and 3 studies were released, which indicated that VN's positive impact on MLMT performance was nearly maximal at 30 days after administration and remained durable for at least 4 years, with ongoing continued observation [36]. Participants included forty subjects who received 1.5×10^{11} vg of VN per eye in at least 1 eye during the trials, including 11 phase 1 follow-on subjects and 29 phase 3 subjects (20 original intervention [OI] and 9 control/intervention [CI] patients who crossed over to receive treatment after 1 year of no treatment).

Mean MLMT score change was 2.4 at 4 years compared with 2.6 at 1 year after administration in phase 1 follow-on subjects (n = 8) [36]. For the phase 3 subjects, mean MLMT score change remained stable at 1.9 between the first and second year post-administration in OI subjects (n = 20), and was 2.1 at 1-year post-administration in CI subjects (n = 9). All 3 groups maintained improvement in FST, reflecting more than a 2 log10 cd.s/m^2 (~100 fold) improvement in light sensitivity at 1 year and subsequent available follow-up visits. The safety profile was consistent with vitrectomy and the subretinal injection procedure, and no deleterious immune responses occurred.

In contrast to the average yearly VF loss of approximately 25 sum total degrees on Goldmann VF III4e in the natural history study of patients with *RPE65* mutation-associated IRD [13], phase 3 subjects had a mean change of +267 sum total degrees at 1-year post-treatment, and OI subjects maintained this increase at 2 years [36]. This denotes an expanded area of retinal sensitivity caused by improved photoreceptor function, which translates into greater light sensitivity and peripheral vision. The improvement in Goldmann VF III4e occurred across a boundary (>500 sum total degrees) associated with

improved performance on the MLMT, as determined by the validation study that described the relationship between MLMT performance and other tests of visual function [39].

Additional 4-year follow-up from the phase 3 study was presented at the 2019 annual meeting of the American Association of Pediatric Ophthalmology and Strabismus, with results substratified by age groups <10, 11–17, or >18 years [41]. There were no significant differences in MLMT performance between any of these age groups in Year 1. Mean changes in MLMT at Year 1 were maintained at Year 4 for the OI group and Year 3 for the CI group (1.7 and 2.4 lux, respectively). At Year 4, 5/20 OI subjects (ages at treatment 4, 6, 11, 11, and 34 years old) showed a decrease of 1 lux. Three of the five remained stable compared to Year 2 or 3. None of the subjects declined below baseline and 1/20 (age at treatment 16 years) gained a lux at Year 4. One subject had a retinal detachment detected in Year 4. The authors suggest that amblyopia is not likely to be a major hinderance to gene therapy treatment, but photoreceptor loss in a progressive disease may limit outcomes.

6. Approval and use of voretigene neparvovec-rzyl in clinical practice

6.1. Approved indication

In December 2017, the FDA approved VN as a treatment for confirmed biallelic *RPE65* mutation-associated IRD, rendering VN the first approved gene therapy for a genetic disease in the US, and the first and only pharmacologic treatment for an IRD [42]. It is not approved for the treatment of RPE65 autosomal dominant RP. Patients must have viable retinal cells as determined by the treating physician in order to be appropriate candidates for therapy. Use in infants under 12 months of age is not recommended because of potential dilution or loss of VN after administration, due to the active retinal cell proliferation occurring in this age group [4].

6.2. Dosage forms and strengths

The recommended dose of VN for each eye is 1.5×10^{11} vg in 0.3 mL, administered subretinally, as in the phase 3 trial [4,9]. The supplied concentration ($5x10^{12}$ vg/mL) requires a 1:10 dilution prior to administration, using the supplied diluent. Treatment is administered to each eye on separate days, no fewer than 6 days and no more than 18 days apart. Perioperatively, oral corticosteroids are recommended, at a dose equivalent to prednisone at 1 mg/kg/day (maximum of 40 mg/day), for a total of 7 days (starting 3 days before administration of VN to each eye) followed by a tapering dose during the next 10 days.

6.3. Ocular gene therapy administration

As with VN, the most commonly investigated method of vector administration to the RPE involves PPV followed by transretinal injection of the viral vector into the SRS. The recommended site for injection is along the superior vascular arcade, at least 2 mm distal to the fovea, and 0.3 ml is injected subretinally [4]. This procedure creates a temporary retinal detachment or 'bleb,' but allows for direct delivery to the RPE. An air-fluid exchange is performed, leaving the eye with an air fill, and the patient is positioned supine in the immediate post-operative period. This air-fluid exchange may minimize reflux of vector into the vitreous cavity, and thus limit any inflammatory response.

For other retinal gene therapies currently under investigation, injection of the vector into the vitreous cavity has been attempted, and although this method may be less invasive and potentially have fewer procedure-related complications, there have been challenges associated with its implementation clinically. Specifically, the internal limiting membrane limits the penetration of viral vector to underlying target retinal layers [43]. Additionally, intravitreal injection is considered more immunogenic compared to subretinal injection [44,45], given the SRS's tight blood-ocular barrier which limits systemic dissemination and exposure of vector to neutralizing antibodies [6,46]. However, techniques such as 'directed evolution' of viral vectors [47] and capsid tyrosine mutations [48–50] may enhance intravitreal delivery and/or minimize host response. Ideally, clinic-based intravitreal administration, and potentially suprachoroidal delivery (only recently under study) [51,52], may circumvent some of the logistical and safety issues of operating room-based subretinal delivery.

Although subretinal administration of gene therapy via PPV represents the most common technique, it is still an evolving procedure with novel facilitating technology including digital visualization systems, smaller subretinal cannulas, precision infusion pumps, and intra-operative optical coherence tomography (OCT) to precisely monitor proper bleb formation. Two of the authors have had extensive experience in the procedure, and the steps of the surgery performed by one of the authors (AMB) are described herein. This approach differs from the protocol used in the clinical development of VN due to the evolving advancements of vitreoretinal surgery noted above; a surgical video can be accessed at this reference [53]. A 25-gauge PPV is completed with the NGENUITY® 'Heads-Up' 3-D Visualization System (Alcon, Fort Worth, Texas, USA). As the majority of the patients are young, a preexisting posterior vitreous detachment is unlikely. After the hyaloid is lifted and a core vitrectomy performed, dilute triescence is injected to ensure that the entire hyaloid is removed. The periphery is shaved with the assistance of scleral depression and examined for retinal breaks.

Prefilled 1 ml BD syringes with the gene therapy product are inspected and mounted on MedOne #3243 high-pressure 6 inch extension tubing with a MedOne #3219 PolyTip 25 g/38 g subretinal injection cannula. The MedOne microinjector system is primed with air first to release the plunger, it is filled with the viral vector and the cannula is attached to the injector. The medication is pushed through the cannula and then the tip of the cannula is trimmed to be beveled to facilitate subretinal penetration. The intraocular pressure is reduced to 10 mmHg, and the tip of the needle is then placed into the vitreous cavity.

Using the flat contact lens (AVI) for increased magnification, an injection site is chosen in the superior macula along the superior vascular arcade at least 2 mm distal to the center of

the fovea. The site should be free of intraretinal pigment migration or dense atrophy. The needle tip is placed in contact with the retinal surface, which may be confirmed with the intraoperative OCT and the automated injector is used. Any contact with the retinal vasculature is avoided. A second bleb may be created inferiorly to allow a more diffuse subretinal injection and to avoid creating a macular hole by overstretching the superior bleb.

The subretinal injection is confirmed with Rescan OCT imaging (Figure 3). The plunger remains depressed for 5 seconds after the syringe empties, and then the needle is withdrawn. The central macula is included in the area of retinal elevation. A total of 0.3 ml of the therapeutic agent is administered. Fluid-air exchange is then performed with an infusion pressure of 30 mmHg, taking care to avoid drainage of fluid near the retinotomy site for injection. Each of the sclerotomies are closed with 7–0 vicryl.

Peribulbar anesthesia with a mixture of lidocaine and ropivacaine is administered followed by a subtenons injection of Triesence (Alcon, Fort Worth, Texas, USA). Subconjunctival injections of cefazolin and dexamethasone are performed, followed by topical antibiotic and atropine drops. The entire procedure is completed within 4 hours of preparation of the therapeutic agent. The patient is instructed to maintain supine positioning immediately after the surgery and for the next 24 hours.

6.4. Pharmacokinetics

Biodistribution of VN was evaluated at 3 months following subretinal administration in non-human primates [4]. Using a quantitative polymerase chain reaction (qPCR) assay, the highest levels of vector DNA sequences were detected in anterior chamber fluid and vitreous of treated eyes. Low levels of vector DNA sequences were detected in the optic nerve of the treated eye, optic chiasm, spleen and liver, and occasionally in the lymph nodes. Vector DNA sequences were not detected in the gonads.

In the phase 3 study, the vector was shed transiently and at low levels in tears from the injected eye in 45% of the subjects [4,9]. Vector DNA was detected in serum in 3/29 (10%) subjects, including 2 with vector DNA in tear samples up to Day 3 following each injection.

6.5. Safety data

Combining the patient pools from the phase 1 and 3 studies yields a sample size of 41subjects (81 eyes) that were exposed to VN, from which safety data can be assessed [4]. Twenty-seven (27/41, 66%) subjects had ocular adverse reactions that involved 46 injected eyes (46/81, 57%), which may have been related to VN, the subretinal injection procedure, the concomitant use of corticosteroids, or a combination of these procedures and products. The most common ocular adverse reactions (incidence ≥ 5%) were conjunctival hyperemia, cataract, increased intraocular pressure, retinal tear, dellen (thinning of the corneal stroma), macular hole, subretinal deposits, eye inflammation, eye irritation, eye pain, and maculopathy (wrinkling on the surface of the macula). The majority of these adverse events were minor in nature, and there were no deleterious immune responses. There were 2 reported ocular SAEs, including 1 event of reduced foveal function assessed as related to the administration procedure, and 1 event of increased intraocular pressure with associated optic atrophy after receiving intraocular antibiotics and periocular corticosteroids for endophthalmitis. As noted above,

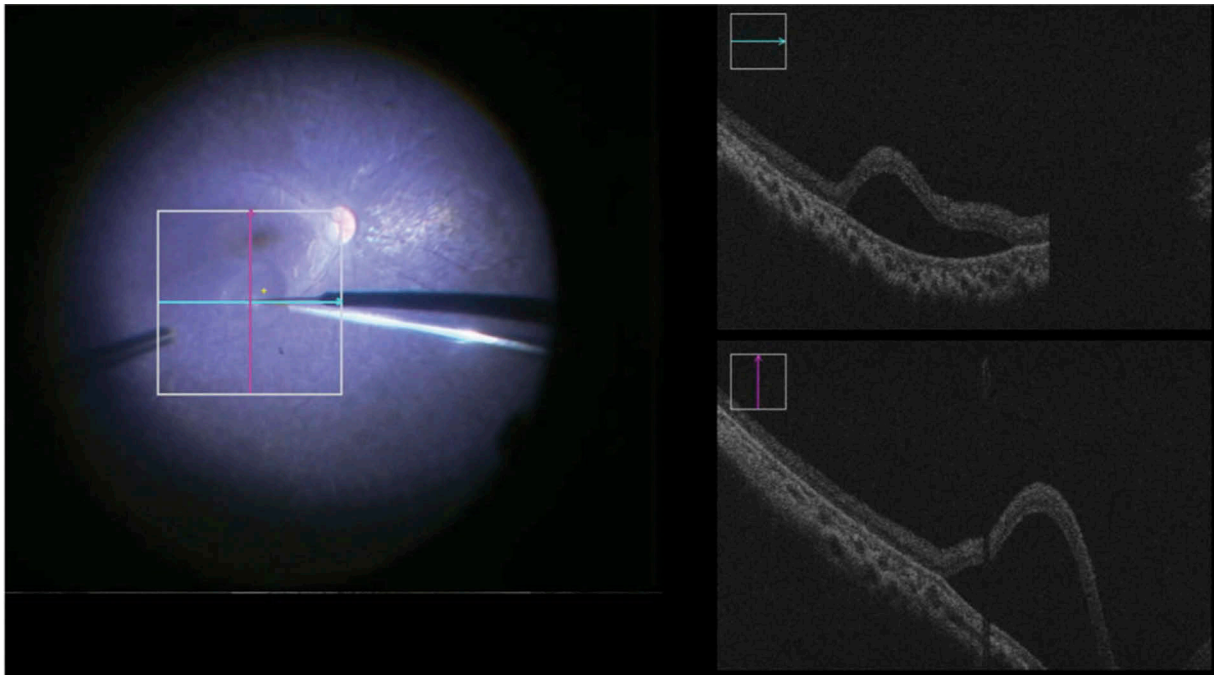

Figure 3. Intraoperative fundus photo and optical coherence tomography captured during the creation of the subretinal bleb. The injection site is chosen by the superior arcade and the central macula is included in the area of retinal elevation. A total of 0.3 ml of the therapeutic agent is administered. Note the optic nerve pallor and severely attenuated retinal vessels, which is typical for RPE65 mutation-associated retinal dystrophy.
This approach, used by one of the authors, differs from the protocol used in the clinical development of VN, and in its prescribing information, due to the evolving advancements of vitreoretinal surgery.

a retinal detachment was noted in a subject 4 years after treatment within the phase 3 study [41]

7. Special challenges associated with introduction of the first ocular gene therapy

In addition to novel surgical complexity of ocular gene therapy administration, special challenges associated with the introduction of VN included limited genetic testing in clinical practice, new functional vision endpoints, as well as unique development, launch, and reimbursement considerations associated with orphan therapies and one-time gene therapies. VN development led to some innovative solutions for many of these issues, which may also surface in future gene therapy development.

7.1. Genetic testing

The limited clinical use of genetic testing in retina practices represented a special challenge in the launch of VN. Prior to the advances in molecular genetics, patients with IRDs generally received clinical diagnoses, and genetic testing was not commonly performed or reimbursed. However, mutations in more than 271 genes are known to cause IRDs [54], and consequently, genetic testing is increasingly important in clinical practice to identify patients who may be candidates for VN or other investigational therapies.

Through a program called 'ID YOUR IRD', Spark Therapeutics initiated and continues to support a genetic testing program, with panel tests for mutations known to cause IRDs [55]. This program enhanced understanding of genetic testing for practitioners, as ordering and interpretation of genetic test results is complex, representing a barrier to adoption of genetic testing. For example, in the phase 3 program, 34 distinct genetic mutations were reported, with 8 subjects showing homozygous mutations and 21 subjects showing heterozygous mutation, but there were no apparent associations between genetic mutation and baseline visual function, treatment response, or adverse events [56].

Furthermore, genetic testing results are not binary and involve a ranking system of each identified mutation based on standards released by American College of Medical Genetics and Genomics (pathogenic, likely pathogenic, variants of uncertain significance (VUS), likely benign, benign) [57]. Pathogenicity is determined by multiple factors, including the effect on gene coding, protein structure/function, variant association with disease in the population and *in vitro/in vivo* functional studies [57]. VUSs can be especially difficult to address. A VUS involves a mutation variant for which there is a lack of affirmative data of pathogenicity or nonpathogenicity, which is especially challenging for patients who would otherwise represent good candidates for VN. The Estimate of Pathogenic Probability (EPP), is another genetic testing scoring system, used by the Carver Lab at the University of Iowa. This system yields values between 0 and 3, with 0 indicating very little probability of disease association and 3 indicating an extreme likelihood of disease association. Values of 1 or 2 have slightly different interpretations, depending on whether the disorder is autosomal dominant or recessive [58]. In some cases, in vitro testing of VUS in *RPE65* may represent the ultimate option to provide evidence of pathogenicity in these situations [59]. Even when pathogenicity of a genetic variation is likely, parental testing (segregation analysis) for patients with compound heterozygous mutations should be considered to confirm the variants are on separate alleles and thus are truly 'biallelic.' These genetic testing and interpretation issues will remain key items to address in future programs of gene therapy for genetic disease.

7.2. Novel functional vision endpoints

As previously noted, the efficacy of VN was established on the basis of the bilateral MLMT [4], developed in response to the need for a relevant, reliable, and clinically meaningful measure of functional vision in patients who were participating in clinical trials [9]. Practitioners have historically relied on VA as the key measure of visual function, but VA is affected late in the course of RP, which primarily affects rods and peripheral vision. Practitioners are less familiar with tests of functional vision, the ability to conduct visually dependent activities of daily living; while the MLMT assesses functional vision, it has been shown to have correlations with VA and VF, as well as visual function questionnaires [39]. Both patients and regulators are particularly interested in functional vision, because activities such as reading or navigating correlate with quality of life.

The MLMT was developed, incorporating feedback from the FDA, to measure ambulatory vision at real-world light levels encountered during activities of daily living; it underwent its own rigorous validation study, which assessed normally sighted and IRD patients over a 1-year time period [9,39]. In the MLMT, patients ambulate through a mobility course under multiple standardized lighting conditions, to determine the lowest illumination under which they can successfully navigate the course in under 3 min. This MLMT was designed to be navigable by children as young as age 3 [39]. In the VN clinical trials, the MLMT was assessed using both eyes and each eye separately at 1 or more of 7 levels of illumination, reproducing the lighting conditions encountered in daily life, ranging from 400 lux (corresponding to a brightly lit office) to 1 lux (corresponding to a moonless summer night) [4]. The MLMT of each subject was videotaped and assessed by independent graders, and the score was determined by the lowest light level at which the subject was able to pass the MLMT [9,39]. The MLMT change score was defined as the difference between the score at baseline and the score at Year 1 [39]. A positive MLMT score change from Baseline to Year 1 visit indicated that the subject was able to pass the MLMT at a lower light level [9]. An MLMT score change of 2 or greater is considered a clinically meaningful benefit in functional vision [4]. These tests of functional vision, such as the MLMT, are now better understood with the approval and launch of VN, and will become increasingly important in the coming decade, as other gene therapies for IRDs undergo clinical trials and potential commercialization.

7.3. Orphan therapy development, launch, and reimbursement considerations

The retina community has generally not been exposed to treatments approved for orphan diseases, which represented another challenge in the development and launch of VN. Historically, rare diseases have been neglected, or 'orphaned,' in drug development due in part to the inherent challenges of lengthy and expensive clinical trial operations in small, often geographically dispersed patient populations. In the US, the Orphan Drug Act of 1983 defined orphan diseases as those that affected fewer than 200,000 Americans [60]. The Orphan Drug Act provided sponsors with 7 years of exclusivity, tax credits to defray the cost of development, waived FDA fees, and provided protocol assistance. The European Medicines Agency (EMA) provided similar incentives in 2000, with orphan designation for products addressing life-threatening or debilitating disorders affecting 5 or fewer per 10,000 individuals [61]. There are additional incentives to develop therapies for rare pediatric diseases; in 2017, Spark Therapeutics announced that it received rare pediatric disease designation for VN [62] and was able to sell the associated priority review voucher in 2018, which provided capital to reinvest back into research and development [63].

Despite these incentives, these small patient populations complicate clinical study, not only through limited trial recruitment but also through limited natural history data. These issues impacted the development of VN for the treatment of biallelic *RPE65* mutation-associated IRD, which necessitated separate studies of natural history, while requiring nearly a decade for phase 1–3 study recruitment and assessment of primary endpoints [13,64,65].

In addition to affecting the development of VN, the small patient populations also complicated the launch of VN and spurred the development of innovative solutions. In general, treatments for orphan diseases can involve unique care pathways because the rarity of the disease often necessitates the centralization of care with multiple providers. For VN, the novel surgical complexity of ocular gene therapy administration, along with the need to prepare and use VN within the recommended 4 hours, created a challenge. Furthermore, there was a need for gene therapy treatment expertise across a team including medical, surgical, pharmacy, billing, and patient support services. To ensure the quality and success throughout the many steps in operationalizing a treatment center, Spark Therapeutics implemented a training program to operationalize 10 geographically diverse treatment centers [66]. This centralized care facilitates safety and treatment optimization because consistent patient and procedural volume in rare disease drives a virtuous cycle of expertise and positive outcomes.

Despite orphan status, the commercialization potential of VN is limited and the business model is challenged. Specifically, while the current health-care system may readily value chronically administered medications, it may not properly value therapies that deliver long-lasting benefits in 1 dose or administration. The value of an intervention generally derives from three major inputs: direct cost offsets (such as lowered cost of care compared to standard treatment), indirect cost offsets (such as lowered societal costs), and impact on quality-adjusted life years (QALYs) [67,68]. These inputs, which greatly affect the outcomes of the resulting cost-effectiveness analyses, are complex and sometimes controversial, especially in determining appropriate indirect costs that address the high societal impact of blindness, or in utilizing appropriately relevant health-related quality-of-life (HRQL) weights (or utilities) to derive QALYs, or in considering potential lifetime treatment effects for one-time gene therapies [68,69].

Specifically, in the US, most commercial insurers may not fully acknowledge indirect cost offsets, since the high societal costs of blindness are generally born by the government. Nevertheless, indirect cost offsets can be substantial for new therapies addressing serious blinding disorders, and relate to increased educational attainment, enhanced productivity, and reduced caregiver burden, as well as decreased reliance on governmental programs. Similarly, payers may not utilize appropriate health utilities to derive QALYs, as the literature has historically assessed visual impairment through VA, derived from studies involving patients with age-related macular degeneration and diabetic macular edema [70,71], with only very recent literature assessing health utilities in IRDs [69]. The profound vision loss in *RPE65* mutation-associated IRD is associated with a substantial impact on health utilities, reflecting potential high value for the restoration of vision by a one-time therapy. Furthermore, for gene therapy, payers are reluctant to model lifetime treatment benefits, given the novelty of gene therapy with its limited long-term efficacy data. Finally, cost-effectiveness analyses are often biased against one-time therapies due to the sequencing of current costs and future benefits, with costs incurred in the short term and benefits distributed over the long term. Specifically, the future benefits of one-time therapies are disproportionately discounted when compared with their current costs [72].

Consequently, new reimbursement models have been considered; these models often involve installment payments and/or tie payment to real-world treatment effectiveness. For VN, Spark Therapeutics developed outcomes-based rebates and an innovative contracting model that supports patient access in the US, while aiming to reduce risk and financial burden for payers and treatment centers. Specifically, Spark Therapeutics offered to share risk with certain US health insurers by paying rebates if patient outcomes (FST testing scores) fail to meet a specified short-term or longer-term threshold, thereby linking the payment for VN to both short-term efficacy (30–90 days) and longer-term durability (30 months) measures [73]. Finally, to facilitate patient access to VN globally, in 2018, Spark Therapeutics entered into a licensing and supply agreement with Novartis for the development, registration, and commercialization rights to VN outside of the US [3]. These programs serve as models to address complex issues surrounding orphan therapy development, launch, and reimbursement consideration.

8. Conclusion

The evolution of gene therapy has been remarkable over the last decade and has proven to be relatively safe in a multitude of small clinical trials, albeit with varying outcomes. Following successful phase 3 clinical trial outcomes, the 2017 FDA approval of

VN, the first US gene therapy for a genetic disease, initiated a new cycle of innovation in ophthalmic gene therapies. VN serves as a model for ocular gene therapy development, while *RPE65* mutation-associated IRD serves as an example of a well-suited candidate disorder. The development and validation of novel functional visual endpoints, such as the MLMT, represent more useful measures of visual benefit in patients with IRDs that predominantly affect rod function, compared to VA, which predominantly reflects cone function. Durability of gene augmentation has been supported by recent publications demonstrating statistically significant improvements in MLMT and FST for up to 4 years after VN treatment. Continued implementation of genetic testing in patients with suspected IRDs is critical to identify those who may benefit from gene therapies. New reimbursement models involving installment payments and/or are tied to treatment effectiveness will continue to evolve. With the lessons learned from the development, approval, and launch of VN, the future is bright for further innovation in ophthalmic gene therapy.

9. Expert opinion

The partnership of industry sponsors with basic science researchers, incentivized by the Orphan Drug Act, has created a supportive environment for gene therapy in IRDs. The success of VN with *RPE65* mutation-associated IRD has stimulated and serves as a development model for, gene therapies for other IRDs, including Stargardt macular dystrophy, choroideremia, achromatopsia, blue cone monochromacy, Usher Syndrome, X-linked retinoschisis as well as Leber hereditary optic neuropathy, an inherited optic neuropathy [5]. However, autosomal recessive *RPE65* mutation-associated IRD may have been particularly well-suited for gene therapy development, as it involves a key enzymatic defect, and augmentation of even a small amount of enzymatic activity can sometimes restore phenotype, while it does not cause irreversible degeneration until later in its course; development efforts also benefited from a large animal model.

Beyond IRDs, there is great interest in gene therapy for VEGF-mediated retinal diseases, such as neovascular age-related macular degeneration (nAMD), for which there are several ongoing clinical trials [74]. If FDA approval is achieved for a mainstream indication such as nAMD or diabetic retinopathy, for which there is a very large global burden of disease, then gene therapy could disrupt current treatment paradigms. Given the invasive nature of PPV with subretinal injection, compared with the current treatment modality of intravitreal injection, there is interest in novel vectors and alternative delivery methods such as intravitreal injection or suprachoroidal injection.

With respect to advanced rod-mediated retinal degenerative disease or in children with dense amblyopia, there is limited utility in using traditional clinical trial endpoints involving VA, with its limited dynamic range and its predominant relationship to cone function; consequently, alternative clinical trial endpoints have been developed. For instance, the results of the VN phase 3 trials demonstrated an improvement in MLMT and FST, but the trend in improved VA did not reach statistical significance. MLMT may more comprehensively assess vision as it more functionally assesses VA, VF, light sensitivity, and mobility in these patients. Furthermore, these novel study endpoints are clinically meaningful to regulators.

FST is a global measure of retinal sensitivity to light. Given that *RPE65* mutation-associated IRD commonly presents with impaired low light sensitivity, FST serves as a relevant visual function test to measure improvement in photoreceptor function. Furthermore, nyctalopia results in decreased ability to perform tasks, including independent navigation, in moderate or low light conditions. The connection between light sensitivity and navigation under low light conditions is proven by the strong relationship found between the post-intervention ability to pass the MLMT at the lowest illuminance level tested (1 lux) and an improvement of FST of >1 log10 units [9,39]. Maguire et al. noted that the ceiling effect associated with the MLMT can partially limit assessment of improvement in functional vision. Pairing the MLMT with the FST overcomes this limitation and corrects for any improvements due to maturation or a learning effect on the MLMT in those in the control group [36].

In addition to efficacy endpoints, other questions remain. Long-term protection against continued retinal degeneration remains unsettled. Although VN was not used, *RPE65* mutant dogs showed rescue from progressive retinal degeneration in the treated zone 5–11 years later, but only when undergoing gene augmentation before the onset of retinal degeneration [75]. Similarly, optimal timing of intervention is unclear. One report from the phase 1 VN study suggested that early intervention could yield best potential gain [34], but this must be balanced against the risks of surgical administration in young children. Nevertheless, the oldest subject in the phase 3 trial did show the median MLMT improvement of 2 light levels at 1 year [9].

In addition, the longevity of gene expression from a one-time gene therapy is still undergoing study, but recently updated results suggest that VN may produce stable improvement in MLMT and FST for at least 4 years [36,41]. Also, it is unclear if repeated treatments to enhance cellular gene expression would be tolerated from surgical administration and/or immune response standpoints. Finally, adoption of gene therapy for IRDs will require widespread genetic screening to identify patients based on genotype, as well as new reimbursement models for expensive one-time therapies that have the potential for lifetime beneficial effects.

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