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Perspective

Perspectives on Gene Therapy: Choroideremia Represents a Challenging Model for the Treatment of Other Inherited Retinal Degenerations

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Purpose: To report combined viewpoints on ocular gene therapy from a select group of clinician scientists and a patient advocacy group.

Methods: With the support of Randy Wheelock and Dr. Chris Moen from the Choroideremia Research Foundation (CRF), a special interest group at the 2019 Annual meeting of the Association for Research in Vision and Ophthalmology in Vancouver, Canada, shared their knowledge, experience, concepts, and ideas and provided a forum to discuss therapeutic strategies for the treatment of inherited retinal disorders, using experience in choroideremia (CHM) as a model.

Results: A member of the CRF presented the patient perspective and role in clinical trials. Five clinician scientists presented reasons for limited long-term visual improvement in many gene therapy trials, including challenges with dose, incomplete understanding of photoreceptor metabolism, vector delivery, inflammation, and identification of patients likely to benefit from treatment.

Conclusions: The shared experience of the five clinician scientists indicates that the results of ocular gene therapy for choroideremia have been less successful than for *RPE65*-related Leber congenital amaurosis. Improvement in vector delivery and developing a better understanding of gene expression in target tissues, treatment dose and side effects, and inflammation, as well as identifying patients who are most likely to benefit without suffering excessive risk, are necessary to advance the development of effective therapies for inherited retinal degenerations.

Translational Relevance: Additional long-term data are required to determine if ocular gene therapy will be sufficient to alter natural progression in choroideremia. Combination therapies may have to be considered, as well as alternative vectors that minimize risk.

Introduction

In sequence, with a single moderator, short presentations at the 2019 Association for Research in Vision and Ophthalmology meeting provided an overview of the current experience of ocular gene therapy using the example of choroideremia. After Leber congenital amaurosis (LCA), choroideremia (CHM) is the next inherited retinal disease for which we have the most experience. Clinical trials of gene therapy in CHM provide important insights into how to obtain,

measure, and evaluate outcome measures of these clinical trials. Not all measures will be equally predictive of change. Treatment of the central fovea is required for the disease stages in the early phases of gene therapy trials in CHM and diseases within the category of retinitis pigmentosa (RP) but presents a significant risk to the remaining visual function. Retinal surgical techniques continue to be refined to minimize trauma and avoid complications and triggers of immunity. Our experience is still not sufficient to know about the sustainability of the current gene replacement protocols in CHM and most other inherited retinal degenerations (IRDs).

Abundant proof-of-concept studies set the stage for reproducible, dramatic, acute restoration of retinal function in clinical trials for LCA associated with mutations in *RPE65* and inadvertently created the expectation in industry and patients alike that a similar outcome was to be expected for the treatment of other IRDs.[1] The *RPE65* gene product plays a critical role in the retinoid cycle that affects visual function before photoreceptor structure in patients with *RPE65* mutations, whereas in many IRDs visual dysfunction results from degeneration or death of the photoreceptors.[2,3] At the disease stages currently being considered for gene therapies in IRDs, a very different scenario from that of *RPE65*-LCA exists, which involves treating residual, relatively preserved central retina necessary for high spatial discrimination and daytime vision, critical for a patient's quality of life.[4]

With no option but to treat the central retina, the risk–benefit ratio equation is drastically shifted from gain of vision from severe vision loss in LCA, for example, to include the potential for loss of useful central vision after treatment in other IRDs. Treatment outcomes for most inherited retinal degenerations are likely to be less dramatic than the acute, sensational increases in retinal sensitivities observed in *RPE65*-LCA after gene therapy. Existing trials are more likely to produce comparatively minor changes in outcome measures and perhaps show instead slowed progression of the disease based on structural and functional parameters. Nowhere is this more clear than in CHM, a condition where end-stage disease leaves minute areas of very abnormal central retina that can still provide excellent visual acuity, even in end-stage disease.

The Patient Perspective

The patient experience will help inform what outcome measures are tractable and practical. This experience can be most important in a phase I (safety) trial leading up to testing the efficacy of an experimental treatment in phase II. The advent of gene therapies for retinal disease has raised hope and actively engaged patients with disorders that, to date, have offered little hope of therapies. Unfortunately, and unintentionally, patients conflate clinical trials with treatments when they are experiments (except for Luxturna). The seminal ocular gene therapy experiments for the *RPE65* form of LCA produced phase I trial reports in 2008[5–7] and culminated with approval by the US Food and Drug Administration (USFDA) of Luxturna as a treatment. The early success of these trials gave impetus to many similar trials of ocular gene therapy. Patient advocacy groups took a leading role in informing patients and families about these trials, driving investment in treatments for rare eye diseases. Some groups maintain registries of patients who agreed to receive updates on clinical trials (e.g., MyRetinaTracker, sponsored by the Foundation Fighting Blindness). Natural history studies serve as a starting point for interventions to define appropriate outcome measures that could demonstrate change in a reasonable timeframe and act as a marshaling platform to queue subjects, identifying those who would be eligible for the clinical trials.

Outcome Measures

With improved understanding of disease mechanisms, several therapeutic approaches are being investigated for patients with different genetic forms of IRDs. As of October 26, 2019, there were over 35 clinical trials listed on www.clinicaltrials.gov investigating potential treatments for IRDs. Evaluating the safety and efficacy of these treatments requires a clear understanding of the natural history of disease progression in patients with IRD to select the most sensitive and reliable disease measures to monitor during the course of a clinical trial. Typically, vision loss proceeds slowly in patients with IRDs, and slow progression presents a challenge to developing treatments. Current standard measures of disease progression monitor visual acuity (VA) and visual field sensitivity, both of which are subjective tests that can be imprecise and unreliable and only indirectly reflect retinal degeneration. They may also be affected by non-retinal factors such as cataract and patient attention during tests.[8] Finding sensitive, objective outcome measures of disease progression and treatment response will speed the development of treatments for these relentless diseases.

The outcome measures used to demonstrate disease progression and patient response to therapy differ depending on the type and stage of retinal degeneration. In early rod–cone degeneration, retinal structure

can be studied with spectral-domain or swept-source optical coherence tomography (OCT)[9] and adaptive optics scanning laser ophthalmoscopy.[10,11] Although VA usually remains normal in early rod–cone degenerations, functional measures including static perimetry[12] and dark-adapted perimetry[13–15] are often abnormal even at early stages of disease. Fundus autofluorescence[16,17] gives information about the health of the retinal pigment epithelium. In moderately advanced disease, the ellipsoid zone (EZ) area reveals the extent of the remaining photoreceptors. Although VA may not yet begin to change,[18,19] conventional static perimetry, dark-adapted perimetry, and full-field stimulus threshold[20] measures are helpful. In later stages of disease, when the EZ area and VA provide limited data to discriminate change, full-field stimulus threshold, mobility tests,[1,21,22] and patient-reported outcomes[23] may reveal altered visual function.

Outcome measures for a given trial should provide mechanistically meaningful metrics validated in the patient group being studied. For this reason, longitudinal natural history studies are necessary to characterize the rate of progression in patients with IRDs and help validate outcome measures for their use in clinical trials.

Sustainability of Therapy and Need for Retreatment

The advent of gene therapy treatment for inherited retinal conditions has come with both success and failure. Although gene therapy interventions designed to treat rod–cone dystrophies have gained USFDA and European Medicines Agency approvals, some outcomes have been less efficacious than expected. The first ophthalmic gene therapy trials were conducted in patients with RP harboring *RPE65* mutations. Improvement in function seen in year 1 was profoundly encouraging, given that phase I safety trials enrolled only patients with severe disease and poor visual function.[5–7] Some clinical trials of adeno-associated viral vector-based gene therapy for *RPE65*-related IRD have reported sustained visual improvements lasting up to at least 4 years after treatment.[24,25] However, other follow-up studies revealed that after 3 years, despite initial improvement in visual function, gene therapy failed to halt or even slow photoreceptor degeneration in these patients.[24–29]

Three hypotheses have been proposed to explain why the results of gene supplementation during long-term follow up may be disappointing for these chronic conditions. First, expression of the delivered gene may have been too low in humans, perhaps as a result of poor transduction due to inefficient gene delivery and/or transcriptional silencing of the therapeutic transgene despite robust transduction.[30] Second, gene therapy may have been given too late; by the time the therapeutic gene was delivered, the photoreceptors may have been so damaged that degeneration could not be halted.[31] An emerging third, alternative hypothesis posits that a congenital imbalance between anabolic and catabolic processes in diseased photoreceptors may limit gene therapy efficacy.[32,33]

Thus far, the literature has found evidence supporting the first hypothesis, refuting the second, and implicating the third. In a significant number of gene therapy cases, the loss of transgene expression can be attributable to host-dependent factors including mRNA degradation and chromatin methylation patterns,[30] suggesting that gene silencing poses a significant obstacle to success. Koch et al.[34,35] found that the *Pde6b* rod dystrophy model is treatable by gene therapy even at advanced disease stages, suggesting that intervention timing does not play a significant role in determining rescue outcomes. In light of these findings, although rods do not have a "point of no return,"[34,35] cones exhibit a selective temporal window that limits the efficacy of neurotrophic therapy.[36] At the same time, recent studies in rod dystrophy models have found evidence for metabolism-induced apoptosis.[37] Disruption in the balance between anabolic and catabolic metabolism occurs when photoreceptors are stressed, limiting the efficacy of gene therapy. Enhancing anabolic metabolism in an RP model slowed photoreceptor degeneration,[37] indicating that photoreceptors may have a congenital metabolic imbalance. Alternatively, a threshold effect may occur such that there is a tipping point, after which the accumulation of toxic metabolites or oxidative radicals may be deleterious to the cells and not corrected by gene therapy. Thus, metabolic reprogramming could serve as a strategy to improve the efficacy of gene therapy.[37–40]

Enhancing the ability of photoreceptors to take up and incorporate nutrients into their biomass (i.e., anabolism) could improve the efficacy of future gene therapies. Interventions that accelerate glycolysis have slowed rod–cone degeneration.[37] Sirtuin6 (Sirt6) is a deacetylase that normally represses the expression of genes that promote glycolysis and anabolic processes.[41,42] The loss of Sirt6, in turn, causes rods to shift toward glycolytic and anabolic metabolism.[37] Blocking this shift toward catabolism, which occurs at a higher incidence in degenerating photoreceptors, can counteract metabolic imbalance.[37,40] Future metabolomic studies may reveal why some gene supplementation interventions have not met expectations and can inform us about timing requirements, which will be critical for optimizing therapies in future clinical trials.

The combination of anabolism reprogramming with gene supplementation therapy[40] will not only enhance the chance of successful gene therapy but also advance the treatment of debilitating retinal degenerations.

Delivery of Vectors and Surgical Challenges

Retinal gene therapy is a complex biological process that depends on multiple factors of which successful vector delivery plays a critical role in determining the safety and efficacy of clinical trials. The two modes of vector delivery in current clinical trials are subretinal[1,43–46] and intravitreal[47–50] administration of adeno-associated viral (AAV) vectors, although suprachoroidal[51] and sub-inner limiting membrane (ILM)[52] approaches are also in preclinical studies. Intravitreal delivery is less technically challenging and has the potential for more widespread retinal gene expression, extending beyond the bleb created by a subretinal injection, as shown in preclinical studies.[53–55] However, due to anatomical differences between the retinas of mouse and primate models, the intravitreal approach may be less effective in humans, especially in treating outer retinal cells. Hence, in primates, the thicker ILM at the vitreo–retinal interphase limits the transduction of cells to a small parafoveal ring even after the injection of novel, mutant capsids with improved cellular transduction in rodents.[56,57]

Intravitreal injection of vector is thus currently limited to clinical trials that target diseases that affect the inner retina, such as Leber hereditary optic neuropathy,[47,49,50] and diseases such as X-linked retinoschisis where the retinal architecture is affected by the causative mutation.[48] Additionally, in X-linked retinoschisis, the disease state arguably breaks down the interphase barriers to some extent, allowing for better vector penetration. Unfortunately, however, no significant gains in visual function were observed in treated eyes compared to controls in these gene therapy studies.[47–50] Moreover, gene transfer from the vitreous of large eyes is highly inefficient due to dilution of the vector when administered into the vitreous cavity. Higher vector doses needed to compensate for this dilution effect and the presentation of the vector itself or of the gene therapy products to immunogenic sites within the ciliary body and anterior structures of the eye increase the risk of ocular inflammation. The vitreous is not an immune-privileged site, and neutralizing antibodies can be induced, reducing vector efficacy and causing unwanted inflammatory reactions.[47,48,58]

The advantage of subretinal gene delivery is that it places high viral loads in direct contact with the target retinal tissue. Unlike the vitreous humor, the subretinal space is immune privileged and able to evade adaptive immune responses.[59] A potential disadvantage is that this technique requires the creation of limited temporary retinal detachment. In advanced stages of retinal degeneration with risks of injury to the retina, this approach can be particularly challenging.[60] Additional difficulties can be encountered in specific disease states (e.g., CHM) where the atrophic retina that surrounds the healthy target island is strongly adherent to the underlying Bruch's membrane. This adherence creates resistance to retinal elevation during the bleb initiation (with risk of vector reflux into the vitreous cavity) and to the horizontal spread of vector within the subretinal space (with risk of excessive retinal stretch and foveal damage).[43,46] In addition, vector reflux into the vitreous cavity reduces the dose applied to target cells and also, depending on the amount of reflux, risks inducing an inflammatory response.[46]

Improvements in surgical technique since the first patients were injected have improved the safety of subretinal vector administration in current clinical trials.[1,43–46] Specifically, to overcome surgical challenges, a two-step technique for subretinal gene therapy has been proposed.[41] This helps to initiate the subretinal bleb with a balanced salt solution during the first step and thus avoid potential vector reflux in cases of difficult detachment. An advancement to this technique uses balanced salt solution mixed with a membrane blue dye (1:50 dilution) to monitor the spread of the solution during the injection. In addition, the dye helpfully stains the retinotomy site, and this same site can then be used to inject the vector in a more controlled fashion during the second step of the injection. In both steps, the injection pressure is controlled by using a foot pedal connected to the viscous fluid injection port of the vitrectomy machine, and the retinal elevation is monitored by real-time intraoperative OCT (Zeiss Rescan 7000, Carl Zeiss Meditec AG, Jena, Germany) confirming the correct tissue plane. Moreover, in cases of extremely thin, atrophic retina, a small amount of perfluorcarbon liquid can be used to prevent detaching the retina in this vulnerable area while still treating the neighboring target island.

In the future, robot-assisted retinal gene therapy may improve the precision and accuracy of subretinal vector delivery beyond what is currently achievable with manual surgery. Initial results of the first-in-human robot-assisted vitreoretinal surgery are encouraging.[61] They have prompted further development of the robotic system to enable slow infusion of the vector solution over a prolonged period to minimize the reflux into the vitreous and reduce the risk of iatrogenic retinal injury.

Treating Residual, Relatively Preserved Functional Retina

Gene augmentation for CHM is being explored in multicenter clinical trials using a treatment scenario that departs significantly from the earlier experience in LCA.[43–46,62–64] In mid- to end-stage CHM, there is no alternative but to treat small, fragile central islands of relatively preserved retina that sustain visual acuity that is often way above the legal limit of blindness, a scenario that is quite different from *RPE65* and other forms of LCA where severely dysfunctional but less structurally fragile retinas are targets for treatment. The resulting shift in the benefit-to-risk ratio is to be expected for the larger group of non-LCA IRDs at the disease stages that are typically considered for initial clinical trials. Results from an ongoing study at the Scheie Eye Institute, University of Pennsylvania, and Massachusetts Eye and Ear at Harvard University assessed the preliminary safety and efficacy data 3 years after subretinal delivery of a recombinant adeno-associated virus serotype 2 (AAV2) vector carrying a human *REP1*-encoding cDNA in CHM patients.[59] Ten subjects with CHM (ages 26–57 years at injection) received uniocular subfoveal injections of low-dose (up to 5×10^{10} vector genome [vg] per eye; $n = 5$) or high-dose (up to 1×10^{11} vg per eye; $n = 5$) AAV2-h*CHM*. Patients were evaluated pre- and post-operatively at study-defined follow up visits for 3 years. Ocular safety was assessed by ophthalmic examination, perimetry, spectral domain optical coherence tomography, short-wavelength autofluorescence (SW-FAF), conventional automated perimetry, and microperimetry. No surgery-related complications or unexpected adverse events were encountered. By 3 years, VA returned to baseline in all but one patient who slowly recovered to –17 letters of baseline. With the exclusion of this patient, mean VA letter count differences (3 years minus baseline) were similar in injected compared to uninjected eyes. Two patients showed greater VA (+5 or 6 letters) in the injected eye compared to baseline and to the uninjected control. Mean sensitivity by microperimetry changed minimally in both injected and uninjected eyes, and there were no significant differences between injected and uninjected eyes in absolute dark-adapted, cone-mediated sensitivities at the fovea.

The modest, borderline significant improvement in VA in a minority of patients in this study echoes previous reports from the ongoing CHM gene therapy trials.[43–46,62–64] Less than 20% of the treated patients, reported thus far, have achieved only modest improvements in acuity in their treated eyes; this emphasizes that, when treating the fovea, we need to understand the complexities and adjust our expectations for success to the disease in question.[65] Therefore, our hope is to establish that treatment efficacy with gene therapy for CHM can be demonstrated when VA remains stable in the treated eyes and represents a true departure from the natural history of the disease. Demonstrating how VA is stabilized or improved in CHM after gene therapy without consistent improvements in foveal function (cone sensitivity, contrast sensitivity, or color vision) remains a challenge for CHM and other IRD clinical trials, as the focus from patients, industry, and regulatory bodies remains the classic measure of change of VA.

Acute (~72 hours) localized foveal cone outer segment shortening and slow, partial recovery of VA in one patient in the University of Pennsylvania/Harvard University study suggest non-vector-related individual vulnerability to the subfoveal injection, an issue reported in at least one subject in each of the current treatment trials.[43–46,60,62–64] Although there is evidence supporting total (9/10) or partial (1/10) cone outer segment recovery over a period of 6 months in the University of Pennsylvania/Harvard University studies, the scenario stresses the need to predict potential damage and protect vulnerable foveas. Similar outcomes may be expected for the larger group of IRDs where a fragile macula is the only region available for treatment via subretinal delivery of gene augmentation products.

Injected and uninjected eyes have shown continued centripetal progression of the sharp transition zones of structural abnormalities characteristic of CHM in all gene therapy trials to date,[43–46,62–64] which may be interpreted as a failure of the treatment to arrest degeneration. Rates of progression have also approximated values observed in natural history studies in CHM that have used SW-FAF and/or the EZ band extent to gauge progression.[66] The reasons why some clinical trials of gene therapy have failed to arrest progression have been debated since the earlier stages of the *RPE65*-LCA trials, where studies reported loss of some functional gains over time.[26–28] The fact that a similar outcome has been observed in CHM is relevant for all gene therapy trials for IRDs. Intraretinal differences in disease severity within the residual islands of relative photoreceptor preservation may explain local differences in the response to treatments, just as inter-ocular or interindividual differences in disease stage may be expected to influence the ability to define appropriate treatment outcome measures[67] or measure significant changes in disease progression. There have been subtle signs of region-specific changes in cone-mediated sensitivity in the University of Pennsylvania/Harvard University CHM trials that hint at that possibility (Aleman TS, et al. *IOVS* 2019;60:ARVO

eAbstract 5173). Longer observation intervals and modification of the outcome measures from averaged measures of sensitivity to location- or region-specific parameters are necessary to better evaluate the significance of these observations and should be the expectation for future studies that will evaluate treatment options for the larger group of IRDs.

Discussion

Retinal degenerations remain among the most challenging diseases to treat in ophthalmology because neural tissue is highly specialized and does not regenerate. Treatment may be more complicated than simply replacing the defective gene, as some studies report that successful gene replacement is less effective over longer periods of time.[27] Effective treatment of retinal degenerations may require a combined approach, including correction of the genetic defect, while adding neuroprotective, immunomodulation, antioxidant, or other mechanisms to sustain and prolong photoreceptor structure and function. Treatment delivery could be improved by modifying current methods of creating a retinal detachment and injecting the treatment into the subretinal space to minimize damage to delicate outer retinal structures.[68] For the moment, the correction of specific genetic defects is most likely to be effective in preserving structure and function, making it critical for patients with IRDs to have genetic testing; however, the genetic cause of degeneration remains unknown for up to 40% of patients with IRD who undergo next-generation sequencing genetic testing.[69–72] For these patients, gene-specific therapies are not an option, but non-specific neuroprotective or anti-apoptotic treatments may be effective in preserving photoreceptor structure and function. Finally, for patients with advanced vision loss, gene replacement may not be effective or lasting when limited cells remain to treat, and visual restoration may require regenerative, optogenetic, or prosthetic approaches. Despite tremendous advances and accomplishments, much work remains to advance the development of treatments for IRDs.

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