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Gene Therapy of Inherited Retinal Degenerations: Prospects and Challenges

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Abstract

Because of its favorable anatomical and immunological characteristics, the eye has been at the forefront of translational gene therapy. Dozens of promising proofs of concept have been obtained in animal models of inherited retinal degenerations (IRDs), and some of them have been relayed to the clinic. The results from the first clinical trials for a congenital form of blindness have generated great interest and have demonstrated the safety and efficacy of intraocular administrations of viral vectors in humans. However, this progress has also generated new questions and posed challenges that need to be addressed to further expand the applicability of gene therapy in the eye, including safe delivery of viral vectors to the outer retina, treatment of dominant IRDs as well as of IRDs caused by mutations in large genes, and, finally, selection of the appropriate IRDs and patients to maximize the efficacy of gene transfer. This review summarizes the strategies that are currently being exploited to overcome these challenges and drive the clinical development of retinal gene therapy.

Clinical Trials of Gene Therapy for IRDs: Success and Challenges Ahead

Inherited retinal degenerations (IRDs), a major cause of severe vision impairment, affect more than 2 million people worldwide.[1] IRDs are a group of diseases with high genetic heterogeneity and differences in inheritance patterns, age of onset, and severity of visual dysfunction.[2,3] Mutations in more than 200 genes mainly expressed in photoreceptors (PR) (Fig. 1B), and to a lesser extent in the retinal pigment epithelium (RPE) (Fig. 1B), cause IRDs.[4,5] Sight-restoring therapy for many IRDs is still a major unmet medical need. However, in the last decades, the identification of many IRD-causing genes has paved the way for the development of gene-based therapies.

The eye has been at the forefront of translational gene therapy because of its small, enclosed structure, immune privilege, and easy accessibility. In addition, the availability of various animal models, along with *in vivo* imaging techniques, allows for noninvasive and consistent monitoring of the effects of gene delivery; outcomes may be compared with disease progression in the contralateral control eye.[6,7] Leber congenital amaurosis type 2 (LCA2) is the first IRD to have been treated with retinal gene therapy in phase I/II clinical trials, the results of which represent the most successful example of ocular gene therapy, to date. LCA2, an autosomal recessive IRD, is caused by mutations in *RPE65*, an essential gene in the retinal pathway. *RPE65* encodes an isomerase protein expressed in the RPE that promotes visual chromophore recycling. LCA2 is an ideal candidate disease for gene therapy because RPE65 deficiency causes defective visual cycle and poor visual function early in life[8,9]; LCA2 retinal structure is left fairly intact until the second to third decade of life, the period in which progressive PR degeneration becomes evident.[8,10] After subretinal administration of a gene therapy vector based on adeno-associated virus (AAV) type 2 (AAV2/2) was shown to induce therapeutic effects in small and large LCA2 animal models, three independent clinical trials were launched (NCT00516477; NCT00643747; NCT00481546). The longest comprehensive follow-up of treated patients reported to date is 3 years.[11,12]

Although it is difficult to directly compare the results of the three studies because of their several variables, data gathered from these clinical trials collectively indicate that gene therapy is both safe and effective.[11,13–19] Patients from all trials exhibited improved retinal and visual function, though to different extents, and reactivation of the visual cortex has been described in one trial.[20] Maximal efficacy was obtained in the youngest LCA2 patients, who

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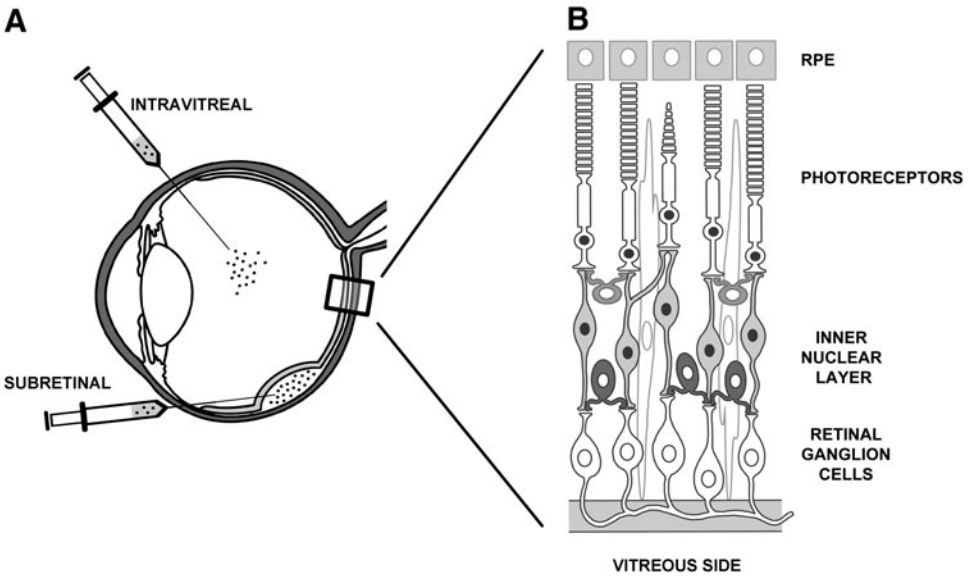

FIG. 1. Schematic representation of intraocular injection routes **(A)** and retinal layers **(B)**.

presumably had better retinal preservation and function to begin with.[17] Importantly, injection of the vector in the contralateral eyes of three LCA2 patients who had been previously treated with the same vector was shown to be safe and effective.[21] This implies that subretinal readministration of AAV2/2 is feasible, even in the case of preexisting immunity to the vector, a criterion that has been used to exclude patients from gene therapy trials involving systemic vector administrations.[22,23]

The biotechnology company Spark Therapeutics is now testing AAV2/2-*RPE65* gene therapy for LCA2 in an advanced phase III clinical trial, a study including patients as young as 3 years of age (NCT00999609). The objective of the study is to file for regulatory approval in the United States as early as 2016.[24] An alternative gene therapy approach for LCA2 treatment is now under evaluation in a clinical trial that uses the RPE65 promoter in combination with AAV2/4 (NCT01496040) that targets transgene expression specifically to the RPE. This may increase the specificity and efficacy of the therapy.[25] Importantly, successful LCA2 clinical trials have encouraged broader application of gene therapy to IRDs due to mutations in genes expressed in various retinal layers, such as *MERTK*, expressed in the RPE and mutated in retinitis pigmentosa type 38 (RP38; NCT01482195)[26]; *ABCA4*, expressed in PR and whose deficiency leads to Stargardt disease (STGD1; NCT01367444)[27,28]; *MYO7A*, expressed in both RPE and PR and found mutated in individuals with Usher syndrome type 1B (USH1B; NCT01505062)[29]; *ND4*, expressed in retinal ganglion cells and mutated in Leber hereditary optic neuropathy (NCT01267422, NCT02064569)[30,31]; and *CHM1*, expressed in multiple retinal cell types and mutated in choroideremia (CHM, NCT01461213).[32] The initial results of the phase I/II CHM clinical trial have been recently published and have confirmed that subretinal administration of AAV2/2 is well tolerated in humans.[32] In addition, recovery of visual acuity and improvement in maximal retinal sensitivity in treated eyes were observed despite retinal detachment.

Although preliminary data from these first clinical trials are extremely promising and bode well for further development of retinal gene therapy, some issues have been raised. Subretinal administration of the vector-containing solution which causes transient retinal detachment[13,16,18] in the LCA2 parafoveal region, in some cases has resulted in permanent retinal damage.[11,18] This raises concerns about using this surgical procedure in retinal tissues diminished by degenerative processes. In addition, in one trial, despite observed visual improvement, the rate of progression of retinal degeneration in the vector-treated retina was reported to be similar to that in the contralateral untreated eye,[33] raising concerns about the longevity of the effects. Finally, unlike what was observed in LCA2 dogs,[25,34–36] no improvement in the full-field electroretinogram has thus far been reported in LCA2 patients treated with AAV. Interspecies differences in either levels of RPE65 expression or AAV retinal transduction efficiency may explain this and suggest that there is room for improvement in LCA2 retinal gene therapy.

Some of the challenges that the retinal gene therapy field is facing after these trials are discussed below along with strategies undertaken to overcome them.

Safe and Effective Delivery of Vectors to the Outer Retina: Are We There Yet?

Subretinal injections, which release the vector into the subretinal space (Fig. 1A), transduce PR and RPE, the two cell types in which the majority of genes mutated in IRD are expressed,[37] most effectively thus far. However, subretinal injections are technically challenging, and concerns about their invasiveness, especially in patients with diseased retinas, have been raised in some of the LCA2 clinical trials in which foveal thinning, macular holes, choroidal effusions, and ocular hypo- and hypertension have been reported.[11,18] Indeed, the interaction between foveal PR and the underlying RPE in the primate retina is very strong; processes emerging from the apical surface of the RPE form a multilaminar sheath that wraps around the outer segment tips.[38] This may explain why foveal reattachment is more complex than extrafoveal reattachment. In fact, subretinal injections in extrafoveal regions exhibiting greater outer nuclear layer thickness than the fovea are being considered in light of the

generation of new ''pseudofoveas'' resulting from vector transduction[15,32,39] (see below). Alternative, less invasive administration, such as intravitreal injection (Fig. 1A), would broadly distribute the vector throughout the retina without causing risky retinal detachment. However, when injected intravitreally, most viral vectors, including the majority of AAV serotypes, do not transduce the retina with the exception of AAV2/2, and to some extent AAV2/6 and AAV2/8, whose transduction is, however, mainly restricted to retinal ganglion and Müller cells in the inner retina.[6,40] The failure of vectors delivered intravitreally to transduce PR and RPE in the outer retina appears to be caused by the presence of physical barriers, such as the inner limiting membrane, which is particularly thick in large animals, as well as the relative abundance of AAV receptors that capture vectors after intravitreal administration.[41] Indeed, if retinal architecture is altered by a degenerative process[42–44] or by enzymatic digestion,[41] the diffusion of AAV viral particles to the outer retina from the vitreous side increases.

Recent efforts have been focused on engineering the AAV capsid to favor its diffusion from the vitreous side. Quadruple and pentuple tyrosine mutant AAV2/2 vectors provided the first proof of concept of the feasibility of outer retina transduction from the vitreous in mice.[45,46] More recently, Dalkara and co-workers used an *in vivo* directed evolution approach to select an AAV2/2 variant (7m8) that was able to transduce mouse PR and RPE following intravitreal injection.[47] However, intravitreal injection of both tyrosine and 7m8 AAV2/2 mutants in larger animal models failed to reproduce the outer retina transduction levels observed in mice, presumably because of more pronounced physical barriers in larger animals than in mice.[47,48] Further understanding of retinal barriers inhibiting transduction as well as *in vivo* directed evolution performed directly in large animals may lead in future to more efficient ways to target the primate retina via diffusion from the vitreous. However, until this is the case, subretinal delivery remains the most efficient administration route to target RPE and PR.

Overcoming the Challenge of Delivering Large Genes to the Retina

Despite the popularity gained by AAV vectors, one of the main obstacles to their widespread application is their packaging capacity of $\sim$5 kb, precluding them from being used to treat IRDs like STGD and USH1B, which are caused by mutations in genes whose coding sequence exceeds 5 kb. Thus, several investigators are exploring alternative vectors with larger cloning capacities than AAV, such as adenoviral (Ad) and lentiviral (LV) vectors, as well as DNA nanoparticles (NP). Ad were the first viral vectors to be tested successfully in the retina.[49] However, Ad vectors efficiently transduce the RPE but less robustly adult mouse PR.[6,50,51] Similarly, LV vectors have been successfully used to transduce PR in newborn rat and mouse retinas and thus, to effectively improve the phenotype of animal models of IRDs.[27,29,52–56] Although recent reports suggest that LV vectors transduce adult nonhuman primate PR,[28,29] RPE transduction has been primarily observed in adult animals.[51,52,57–60] The thicker and more developed physical barriers, which are found in adult as opposed to newborn mouse retina, have been hypothesized to limit adult PR transduction by large particles such as Ad and LV.[61–63] Indeed, the efficiency of PR transduction in adult animals can be improved by enzymatic disruption of the inter-PR matrix or by advanced retinal degeneration.[61–63] Despite the drawbacks of LV for PR transduction, two recent phase I/II clinical trials in STGD1 and USHIB patients (NCT01367444 and NCT01505062, respectively) may provide alternative platforms for gene therapy of IRDs caused by mutations in large genes. Nonviral vectors with large cargo capacity, such as polyethylene glycol-substituted 30-mer lysine peptides (CK30-PEG)-compacted DNA NP, have been shown to safely deliver genes, including those exceeding 5 kb in size, to PR and RPE.[64–67] Thus, compacted DNA NP are a potentially viable option for delivery of large genes to PR. However, limited experience with these vectors, along with the need to clarify their transduction characteristics in large animal models, including nonhuman primates, prompts further testing before compacted DNA NP can be used to deliver large genes to human PR.

As an alternative platform has yet to convincingly match the PR transduction ability of AAV, considerable interest has been directed toward expanding AAV cargo capacity. Efforts at packaging large genes into single AAV particles have resulted in the generation of oversized AAV vectors that, although able to transduce large genes in PR,[68,69] contain genomes highly heterogenous in size,[70–74] potentially posing a major safety concern for their further clinical development. Alternatively, the inherent ability of AAV genomes to concatemerize[75] has been exploited to generate dual AAV vectors, each containing one of two halves of a large gene expression cassette. Different dual AAV strategies (referred to as trans-splicing,[76] overlapping,[77] and hybrid dual-vector strategies[78]) have been used to efficiently deliver large genes to various tissues, including the retina.[76,78–88] Various groups have independently reported that dual AAV trans-splicing and hybrid vectors efficiently reconstitute large genes in mouse[81,82,89,90] and pig PR.[82,83] Although the levels of PR transduction achieved with dual AAV vectors are lower than those achieved with a single AAV vector ($\sim$4% in mice and 40% in pigs[82,83]), subretinal administration of dual AAV vectors has been shown to significantly improve the phenotype of mouse models of STGD1 and USH1B.[81,82] Current efforts aim at improving the levels of transgene expression from dual AAV while reducing those of shorter proteins produced from either the 5′ and 3′ half vector of dual AAV.[82,90] Considering the low levels of PR transduction achieved by vectors with high cargo capacity and the improved cargo capacity offered by dual AAV vectors in the retina, they may be the preferred platform for delivery of large genes to PR in IRDs.

Suppressing and Replacing Rhodopsin: Easier Said than Done

One-third of IRD patients with a recognizable pattern of inheritance are affected by dominant forms of the disease.[2] Many cases are because of mutations resulting in toxic gain-of-function effects.[3] In these cases, reducing the toxic product rather than adding a correct copy of the gene is required to provide significant benefits. Given that more than 150 different mutations associated with dominant retinitis pigmentosa have been described in the rhodopsin

(*RHO*) gene alone, most of the gene therapy efforts to date have been directed to silence *RHO* mutations. Allele-specific catalytic RNAs, including ribozymes[91,92] and short hairpin RNA,[93] have been explored with variable degrees of efficacy. However, the most efficient silencing has been obtained by targeting both the wild-type and mutant *RHO* alleles.[94–100] As doing so results in robust *RHO* suppression, the simultaneous addition of a *RHO* copy resistant to silencing is required as part of the so-called suppression and replacement therapeutic approach to dominant IRD. Alternatively, zinc-finger transcriptional repressors have been designed to silence *RHO* at the level of its locus,[101] a strategy that may theoretically overcome the challenge of silencing this very abundant protein that accounts for >70% of the total rod PR outer segments protein content.[102] New-generation customized DNA-binding modules, including TALE and CRISPR-inactive CAS9,[103] provide new tools to design effective *RHO* transcriptional repressors. While *RHO* expression has been successfully suppressed to therapeutically relevant levels in animal models by several groups,[94,97,98,101] its replacement to sufficient levels (at least 50% of endogenous) appears challenging given *RHO's* high expression levels in PR. However, this is necessary to avoid converting an often mild dominant disease into a more severe recessive condition.

The Importance of Selecting the Right Disease and Patients

Optimal vectors and delivery routes do not guarantee the success of gene therapy. As gene transfer efficacy relies on viable target cells, identifying patients early enough in the course of their condition is crucial to reap the benefits of gene therapy. Conditions like LCA2, LCA1,[104] or achromatopsia[105] exhibit preserved retinal structure for decades after the diagnosis despite severe visual impairment. These forms could be considered as ideal candidates for gene therapy, not only from a therapeutic perspective, but also from a clinical development standpoint, as the rescue of the functional component of the disease can be tested in a reasonable time frame in the context of clinical trials. Instead, many forms of retinitis pigmentosa show mild phenotypes because of a slowly progressive retinal degeneration. While from a therapeutic perspective these are also good targets for gene therapy, given the wide window of time for therapeutic intervention, clinical development of novel therapies for such conditions appears challenging because preventing degeneration becomes the main endpoint of clinical trials; this requires long-term observation and a detailed knowledge of the natural history of the disease. For many conditions that exhibit quick degeneration in combination with a functional defect (i.e., STGD1 in which transport of retinal is impaired,[106] or LCA4 in which key enzymes in the phototransduction cascade are destabilized[107]), early gene therapy may be predicted to both prevent retinal degeneration and restore visual function in transduced PR, which could be addressed in a reasonable time frame in the context of clinical trials. Incidental findings in LCA2 and CHM clinical trials that perimacular areas transduced by viral vectors are converted into functional ''pseudofoveas''[15,32,39] are a promising sign that gene therapy can be effectively applied to conditions with advanced stage degeneration should areas with spared PR still be available. In summary, timely intervention, which requires early clinical and molecular diagnosis in combination with a well-characterized natural history of the disease, will be required to maximize efficacy of gene therapy for IRDs. Ultimately, retinal gene therapy is more likely to reach the final stages of clinical development for those conditions deriving from a functional defect that can be reverted by gene delivery than for purely degenerative diseases in which efficacy of gene delivery is predominantly determined by the extent of prevention of further degeneration.

Future Considerations

Recent advancements in high-throughput genotyping techniques for accurate noninvasive *in vivo* monitoring of retinal and visual function and development of safer and more efficient vectors for retinal gene therapy are factors improving our ability to treat genetic blindness. With these approaches we are able to (1) diagnose conditions with the highest genetic heterogeneity in humans at the molecular level, (2) provide an early and accurate picture of the retinal statuses of these patients, which can be followed up over time, and (3) use gene transfer tools with maximal therapeutic efficacy and minimal local toxicity. The next challenge for the widespread application of gene therapy to many IRD is one that involves funding and infrastructure for clinical translation. Research funding is always a challenge, especially for rare diseases. While preclinical studies can generally be funded through traditional avenues, clinical translation needs more substantial investments for clinical-grade vector production, nonclinical safety testing, regulatory filing, and clinical studies. U.S. and E.U. funding agencies are increasing available funding allocated to support the development of new therapies for rare diseases. Growing budgets are prompting the establishment of collaborations between academic centers, where initial preclinical proof-of-concept studies are performed, and industries, which nurture clinical development by providing the appropriate infrastructures that are required for market approval. On the other hand, biotechs and pharmaceutical companies are beginning to look at rare diseases and gene therapy with more interest than ever before, as the latter has reached the first clinical successes and market approval, and the former are ideal targets to test the safety and efficacy of novel therapeutic platforms. Yet, to develop and test a gene-based treatment for each and every one of the hundreds of different genes involved in IRDs would not be feasible, especially for those rarely mutated in patients. Standardizing production and reagents for those vectors most commonly used in clinical trials has been proposed as a first step in improving the efficiency of translational research.[108] In parallel, data sharing (including cross-reference of Investigational New Drug application/Investigational Medicinal Product Dossier) from pharm/tox studies and long-term follow-up in large animal models may promote more efficient development of new approaches.[108] In conclusion, translating retinal gene therapy from animal research into clinical trials is still a lengthy process, but the field is actively working on defining the missing points that will allow translation of many promising approaches from bench to bedside.

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Author Disclosure Statement

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