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Commentary

Retinal Gene Therapy Coming of Age

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About 5 years ago, results were made public from three independent early phase clinical gene therapy trials for an inherited and early onset form of retinal blindness called Leber congenital amaurosis due to RPE65 deficiency (LCA2) (Bainbridge *et al.*, 2008; Cideciyan *et al.*, 2008; Hauswirth *et al.*, 2008; Maguire *et al.*, 2008). These studies were arguably a first for an *in vivo* gene therapy approach to convincingly deliver both on safety and efficacy. Indeed, subjects suffering from a progressive loss of vision due to this retinal dystrophy regained moderate yet significant improvements in light perception and more complex vision. The binary hallmark of the pathology of LCA2, as well as of most other hereditary retinal blindness, is a combined dysfunction and degeneration of photoreceptor cells (Bramall *et al.*, 2010; Hartong *et al.*, 2006). For these retinal dystrophies with autosomal recessive inheritance, the hope and anticipation was that supplementation of the wild-type cDNA of the disease gene would ameliorate both the dysfunction, resulting in improved vision, and the degeneration, leading to preservation of photoreceptors. In the first reports from the LCA2 trials, vision tests were primarily used to evaluate efficacy. However, a recently published follow-up study by Cideciyan *et al.* (2013) studied both aspects of the disease in subjects enrolled in one of the three clinical trials. Remarkably, the authors found that while gene therapy indeed leads to a long-term improvement of vision, the photoreceptor cell layer continued to decline and gene augmentation effectively leaves the degenerative processes undisturbed.

Cideciyan and others (2013) first performed a thorough study of the natural history of the disease, which provided essential data for interpreting the findings from the treated patients. They used a key measure of retinal degeneration, the thickness of the photoreceptor layer, referred to as the outer nuclear layer (ONL). Improvements in imaging technology over the last decade now make it possible to noninvasively monitor ONL thickness by optical coherence tomography (OCT), which can provide retinal cross-section images at high resolution. OCT has allowed for longitudinal evaluations of retinal degenerative disease processes, as well as the impact of experimental therapies. Once the natural history of RPE65 disease was established, these data served as a control against which the degeneration of the treatment group was compared. In addition, two other clever controls were included: one using the untreated control eye and another using untreated retinal regions of the injected eye. The results showed that "the great majority progressed along the expected natural history, with rare regions showing no change or greater than expected deviation" (p519). This finding became even more striking in light of the fact that visual improvement in these subjects was substantial (on average 1.6 log units) and persistent over time (up to 4.5 years).

To frame these sobering data, the authors reached back to the animal model that originally put LCA2 gene therapy on the map: the naturally occurring dog model of RPE65 disease. Natural history studies of the canine form of inherited retinal dystrophy due to RPE65 were also done. A comparison of the degeneration of the ONL in dogs with that in humans showed an interesting difference. In humans, dysfunction is always accompanied by degeneration; however, RPE65 mutant dogs can have deteriorated vision early in the disease in the absence of photoreceptor degeneration. Only later in life, after about 5 years, ONL thinning becomes apparent and the disease course in dogs becomes similar to that observed in humans. Importantly, gene therapy in the early phase of the disease in dogs, prior to the onset of degeneration, was able to stem the degeneration of photoreceptor cells. However, as with humans, treatment of dogs after degeneration had begun did not slow down photoreceptor loss.

The authors conclude that there appears to be a threshold of accumulated changes as a result of the genetic lesion in RPE65, after which photoreceptor death is inevitable, even following reconstitution of the RPE65 in the therapeutic target. These findings have implications for other inherited forms of retinal degeneration and the design of gene therapy interventions, as indeed in most preclinical studies for other blindness genes, photoreceptor degeneration in animal models can only be warded off when treated prior to the onset of disease. The authors therefore call for a refinement of the current therapeutic approach to enable a more complete therapeutic outcome. One suggested strategy is a

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combined gene augmentation and neuroprotective therapy. Of course, another obvious change would be treatment earlier in the disease course, prior to the point of no return. While the models in the current study predict there is no such therapeutic window in the human course of RPE65 disease, one test of this prediction is currently underway in another RPE65 clinical study in which more subjects of younger age were enrolled (Maguire *et al.*, 2009). Future studies are warranted to define such a window for other forms of inherited retinal degeneration.

Where does this leave the field, and where do we go from here? After the excitement of the results in these pioneering studies, retinal gene therapy has gained momentum toward the clinic. Do these data lessen the excitement and give cause for a slowdown in translation in this arena? In what areas do future therapies need to seek refinement in order to avert these apparent unstoppable degenerative processes?

To address these questions, and to move forward from the important issues raised by Cideciyan *et al.* (2008), it is critical to better understand the nature of the death processes that lead to the apparent point of no return highlighted in this study. The degeneration in the retina of RPE65 patients may be similar to that seen in other retinal diseases where there is a non-autonomous cause of death. For example, in the disease known as retinitis pigmentosa (RP), cone photoreceptors die, even though only rod photoreceptors express the disease gene. RP cones are fully functional until the majority of rods die, at which point the cones become dysfunctional and subsequently die (Hartong *et al.*, 2006). Similarly, studies of genetic mosaics in mice, in which wild-type rods were intermingled with mutant rods, showed that wild-type rods died with kinetics similar to those of the mutant rods (Huang *et al.*, 1993; Kedzierski *et al.*, 1998). There have been various models made to explain why there is nonautonomous death of photoreceptors including a toxin released by dying rods, lack of a growth factor made by healthy rods, oxidation, and metabolic problems with the cones after the rods die (Punzo *et al.*, 2012). However, there is currently a lack of understanding of the disease processes. Nonetheless, there is a possibility that one can provide a nonspecific tonic for ailing photoreceptors, along the lines of a growth factor or a metabolic booster. Indeed, delivery of a growth factor to RP eyes is in a clinical trial, in which an implanted device containing cells that secrete ciliary neurotrophic factor is being used. If successful, it is possible that a growth factor and a prosurvival agent can be combined with a specific gene therapy aimed at correcting the functional deficit (Sieving *et al.*, 2006).

While it is clear that the biology of degeneration in inherited retinal dystrophies uncouples from the primary genetic etiology at some point in the disease progression, we argue that more sophisticated methods and approaches of genetic therapeutic intervention may push back this point of no return. Neither dogs nor humans treated following onset of degeneration demonstrated a reduced rate of photoreceptor loss; however, we do know gene delivery in these studies could be more efficient or be distributed more evenly across the retina with novel technologies. In addition, expression of RPE65 was driven by a promoter with little or no cell specificity, resulting in ectopic expression with possible deleterious effects. Arguing against this possibility, however, is the lack of degeneration observed in the dogs treated as puppies; although there may have been compensation by the younger, less degenerated environment of the young dog. In addition to effects of the transgene, the invasiveness of the subretinal injection, as well as the limited area of the retina that can be reached with this surgical route of administration, may be well tolerated in a nondegenerated retina, but once degeneration has set in, it might be one insult too many. Less invasive and more broadly distributed, yet equally efficient delivery, may overcome this. It is curious, however, that there was not a more immediate increase in the degeneration rate if indeed the injection method was a contributing factor. In short, technical advances may enable improvements on the current approach and allow for later intervention that slows down retinal degeneration (Vandenberghe and Auricchio, 2012).

Whether these data diminish the results that came out of the initial RPE65 trials is left to the beholder; however, it deserves to be restated that these pioneering studies met the primary tenet in medicine—first, do no harm—and went beyond by providing real-life clinical benefit in improvement of vision. It would have been surprising if the first version of a highly novel therapeutic paradigm was flawless and did not require improvement. In fact, the excellent study at hand demonstrates how essential human data are in the development of early stage therapeutic platforms because these first clinical studies provide benchmarks and guideposts for recalibration in case of failure and refinement in all other cases. In our view, clinical translation should progress cautiously, without interruption, to help current generations of patients. The data from human studies, improvement in the therapeutic methods, and a deeper understanding of the underlying disease processes, can each inform the other. This will be an iterative process, hopefully with an increase in the prolongation of vision at each step.

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