The Evolution of Retinal Gene Therapy: From DNA to FDA

BY JEAN BENNETT, MD, PHD; AND ALBERT M. MAGUIRE, MD

The Gertrude D. Pyron Award was created by the Retina Research Foundation to recognize outstanding vision scientists whose work contributes to knowledge about vitreoretinal disease. At the American Society of Retina Specialists 2011 Annual Meeting, the Pyron Award recipients were Jean Bennett, MD, PhD, and Albert M. Maguire, MD, whose pioneering work with retinal gene therapy is ongoing at the University of Pennsylvania and the Children's Hospital of Philadelphia. The husband-and-wife team shared the privilege of delivering the Gertrude D. Pyron Award Lecture, titled "The Evolution of Retinal Gene Therapy: From DNA to FDA." Highlights of the award lecture are summarized in the following article.

JEAN BENNETT, MD, PHD

There is currently no US Food and Drug Administration (FDA)-approved gene therapy product in the United States. However, genetic research continues to grow. It may be that early successes in ocular gene therapy may lead the way for all sorts of gene therapies and to more widespread research in the field.

Decades of scientific developments have led to the prospect of performing retinal gene therapy in humans. These developments include the identification of the structure of DNA by Watson and Crick in 1953, the unraveling of the genetic code, the ability to sequence DNA and to clone it using polymerase chain reaction (PCR), and of course the monumental work of the Human Genome Project that has ushered in a new era of genetic science. All of these developments led to the ability to identify genes associated with diseases of the retina. The first 2 genes for retinal diseases were identified in 1990, for choroideremia and for a form of retinitis pigmentosa (RP), the rhodopsin gene. Subsequently, many more have been identified.

Our own work depended on the identification of the human RPE65 gene in the late 1990s,[1] and the subsequent identification of the canine RPE65 gene.[2] The first gene therapy for a retinal degeneration was initiated in 2007; that trial was completed, and the results were published in 2009 by Maguire and colleagues.[3]

What happened over time to allow this to take place? In 1985, the first transfer of human genes was reported.[4] I had the opportunity of working with the senior author of the report, W. French Anderson, MD, a few years before that publication. Later, Al and I discussed whether it would it be possible to use gene therapy to treat a retinal disease. In 1990 we performed the first retinal gene transfer in vivo in a large animal.[5] Although we were pleased with the results of this study, we found that the transferred reporter gene stayed active for only about 2 weeks.

The tools to allow long-term gene expression, which did not exist at the time of that work, evolved over the next decade with the development of recombinant viral vectors. These vectors could be used to deliver genes into the retina, specifically to either the photoreceptors or the retinal pigment epithelium (RPE) via subretinal injection. We demonstrated this using the same reporter gene with a recombinant adenovirus.[6]

We subsequently demonstrated the first proof of concept of retinal gene transfer in a mouse model of RP.[7] In the years that followed, Al and our colleagues developed techniques to deliver genes safely and stably to the canine retina. Simultaneously, a number of vectors were developed, and today we have an impressive vector tool kit that allows us to deliver genes specifically to designated cells in the retina.

These tools then gave us the opportunity to test gene therapy in Leber congenital amaurosis (LCA), a rare, autosomal recessive condition with very early onset visual disability, in infancy. The gene responsible for LCA was iden-

tified in the dog model to mirror human LCA. In LCA there is progressive degeneration over time in multiple visual parameters, including visual fields, and all individuals, whether dogs or humans, have flat electroretinograms (ERGs). This therefore seemed like an ideal opportunity to test gene therapy, and we tested it first in the dog model.[8] In that work, visual function was restored in this large animal model of childhood blindness.

The scenario for gene therapy in this model was relatively simple. The RPE is the location of an isomerhydrolase called RPE65, which helps to provide 11-cis-retinal to the photoreceptors. Without the normally functioning RPE65 gene in the RPE, no vitamin A is delivered to the photoreceptors, and therefore vision is damaged. Gene therapy was used to deliver the normal copy of RPE65 and overcome the deficit.

In the canine model, after delivery of gene therapy the uninjected eye showed no pupillary light reflex, whereas the injected eye showed a brisk pupillary response. We carried out studies in some 60 dogs and an approximately equal number in a mouse model. We found in all cases that a single subretinal injection in young affected animals led to stable expression of RPE65 and reversal of blindness. Younger animals showed a better response. The expression was localized to the region of the retina that was targeted, and there was a high degree of safety with this approach.

With promising results in several animal models, the next question was, How do we get to a clinical trial? In July 2005, Katherine A. High, MD, of the Children's Hospital of Philadelphia (CHOP), Center for Cellular and Molecular Therapeutics, invited me to be Scientific Director of a pediatric clinical trial of this potential therapy for LCA. Al and I were thrilled at the opportunity because CHOP had assembled a world-class team of investigators to assist in conducting the trial.

Al will now continue with the rest of the story.

ALBERT M. MAGUIRE, MD

So, we had a form of childhood blindness, LCA, for which we had shown successful treatment in animals with the same genetic condition. We now wanted to treat humans. What we encountered next is what they do not teach you in medical school. Getting a drug approved for human use is a highly stereotyped process dictated by federal regulations. It is usually undertaken by drug companies, not scientists, and it is much more akin to accounting than to science.

The first step is to get Investigational New Drug (IND) status from the FDA. This means safety studies must be done in animals, and for these purposes the efficacy studies you have performed up to now are entirely irrelevant. Biodistribution studies are done in normal animals. Testing is performed by independent contractors who validate all equipment and all procedures. They also oversee the chain of custody of the drug being tested.

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Regarding the safety of the injection procedure itself, there is evidence of focal damage at the injection site, but only on histopathology. Clinical experience shows that subretinal surgery is compatible with functional improvement and good visual results.

The next step toward a gene therapy trial was to get approval from the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health. The main issue for our study was that we wished to enroll children. From an ethics perspective, children are a vulnerable population, and in a phase 1 study the preference would be to treat adults first. We argued that LCA is a pediatric disease, and the target population is children. By adulthood there may be no way to measure toxicity.

We also had ample data from our laboratory studies showing that treatment success was much greater in young animals. In our canine model we had success only in animals younger than 18 months, and in my hands I was not able to raise a bleb in animals over that age because of the scarring down of the retina. In mice we had a greater than 80% surgical success rate injecting animals younger than 4 months, vs less than 15% at 2 years of age. Clearly, then, there was a prospect of benefit for children. We were not looking solely at safety.

Ultimately the calculus of ethics depends on the risk-benefit ratio. There may be additional risks, such as the development of amblyopia in children, but the chance of deriving benefit is much greater than in an adult with a scarred-down retina.

We received unanimous approval from the RAC for our proposal. So then we were free to proceed—after receiving final approvals from the FDA, two institutional review boards (IRBs), our institutional biosafety hazard committee, device committees, and so on.

The study followed a dose-escalation design.[3,9] Twelve eyes of 12 patients were treated in 3 cohorts at 3 dose levels. The CHOP IRB insisted that for ethical reasons the lowest dose have some prospect for efficacy based

on our animal studies. Subjects were legally blind based on visual acuity or visual field.

In the procedure itself, 0.15 to 0.3 mL of AAV2.hRPE65v2 was injected into the subretinal space of the macular area using a 39-gauge hydrodissection cannula. With 0.3 mL, the largest dose, the bleb covers about one-fifth of the total area of the retina and is absorbed in as little as 6 hours. During 1 injection early in the study, a fistula developed, which dehisced the fovea, and since then we have used perfluoro-octane liquid to buttress the macular area. We have also moved the injection site further from the foveal center.

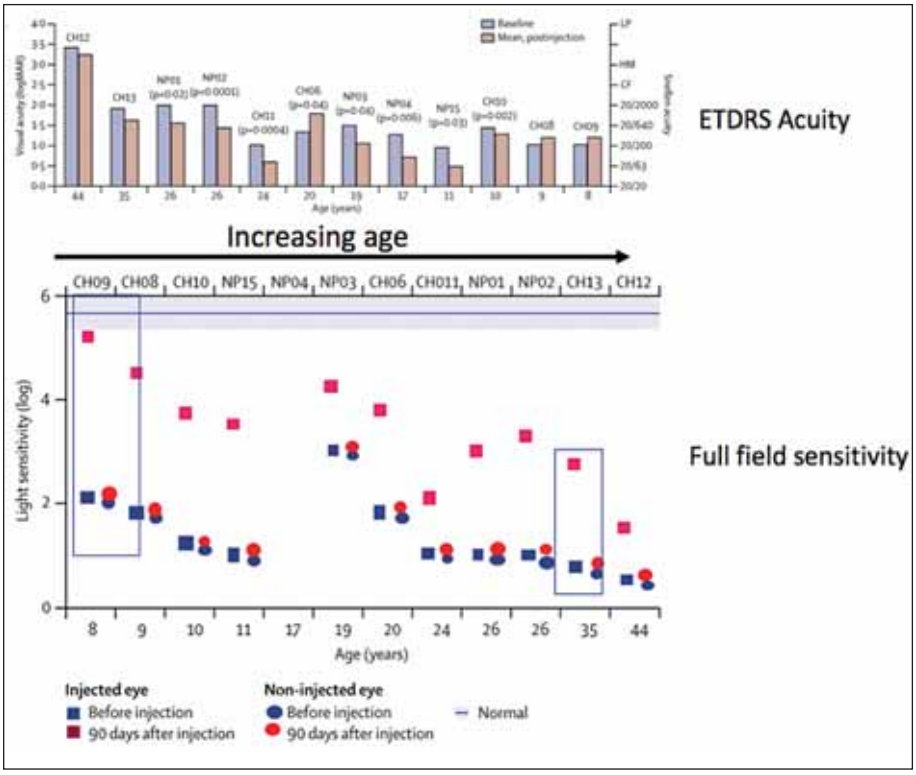

Figure 1. In some of the younger patients, photoreceptor function improved over 10 000-fold, nearly to normal.

To establish efficacy we evaluated multiple outcome measures. Subjective tests traditionally used to measure vision, such as visual acuity or dark adaptometry, are sensitive but vulnerable to a learning placebo effect. We used multiple objective measures, including ERG, pupillometry, and optical coherence tomography (OCT), to corroborate results from these visual tests.

Although visual acuity improved in about half the patients, there was a much greater and more consistent improvement in light sensitivity. In some of the younger patients, photoreceptor function improved over 10 000-fold, nearly to normal (Figure 1). In real-world terms, this is

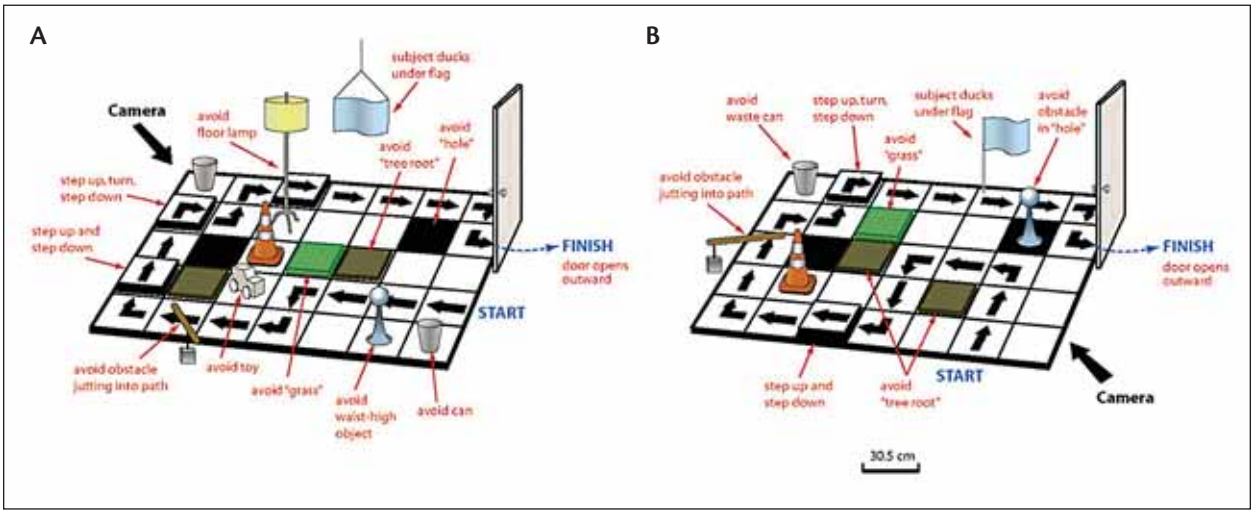

Figure 2. A mobility course was devised to test subjects' ability to ambulate through a room under normal lighting conditions. The course can be changed for each run so that patients cannot memorize the layout kinesthetically.

the difference between being totally blind and seeing well enough to be able to walk through a room. There was no change in the untreated eyes.

Most objective tests such at ERG and OCT are not sensitive enough to detect function in patients with severe retinal degeneration. However, most LCA patients have intact pupillary light response at some level of light intensity, even in advanced disease. We took advantage of this pupillary response in assessing outcomes. We reasoned that, if we could create an afferent pupillary defect in the untreated eye, the conclusion would be that the treated eye must have improved retinal function. We observed that after treatment, the pupils react when the treated eye is flashed, and when the light is moved to the other eye they dilate, an afferent pupillary defect in the untreated eye.

In collaboration with Manzar Ashtari, PhD, at CHOP, we examined whether treatment correlated with brain activity using functional MRI. We found an exquisite correspondence between the area of retina injection, visual field expansion, and activation of the visual cortex.[10]

To assess whether this procedure provides useful vision, we devised a mobility course to test subjects' ability to ambulate through a room under normal lighting conditions (Figure 2). The course is changed for each run, so patients cannot memorize the layout kinesthetically. In a typical result, a young patient takes more than 5 minutes to navigate the course with his treated eye patched. Using the treated eye, he takes a mere 19 seconds.

In other aspects of daily living the treatment also provides benefits. A child who before treatment required assistance ambulating and relied on Braille to read and write, after treatment no longer needs Braille and is able to attend a regular school. His only restriction is that he needs to sit in the front row.

Where do we go from here? First we want to know whether we can treat these patients' fellow eyes. Remember, vectors are viruses, and therefore they are antigenic. Even though the eye is immunologically privileged, there might potentially be an immune reaction if the first injection served as a vaccination against the viral vector. We have treated the second eyes of 3 subjects and have not seen any adverse response. In addition, all three patients showed not only preservation of the gene therapy rescue of the previously treated eye but also a robust therapeutic effect in the second eye [article in press].

Our ultimate hope is to make this therapy available to all patients with LCA. To do this, we have to prove that the treatment is clinically meaningful: that is, that it has a positive impact on the activities of daily living. This is a federally mandated requirement, and we believe it is achievable. The challenge is that visual acuity, which is the one measure identified as being clinically meaningful, is of secondary importance in this mainly rod-mediated disease. Several groups, including ours, are working with the FDA to develop an outcome measure that satisfies the "meaningful" requirement and that can be applied to our target population, children. We hope to begin a phase 3 trial in collaboration with investigators at the University of Iowa in the near future.

In closing, Jean and I feel strongly that we are accepting this Gertrude D. Pyron Award on behalf of our entire group of collaborators at CHOP and the University of Pennsylvania. We would also especially like to recognize the patients who volunteered for this study. Imagine risking what little vision you have to be part of a gene therapy experiment. These individuals are the real heroes of this story. ■

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Both authors are coinventors on a pending patent for retinal gene therapy. They have waived any potential financial gain from the patent. Dr. Bennett served as a scientific advisory board member for Sanofi-Aventis and Avalanche Technologies.

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