My Career Path for Developing Gene Therapy for Blinding Diseases: The Importance of Mentors, Collaborators, and Opportunities

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I WOULD LIKE TO THANK the members of the selection committee of the Human Gene Therapy Pioneer Award for this honor. Truly, the experiences I have had in science are reward enough. I never could have dreamed of having such a gratifying career. As I talk to students and mentees about their career plans, I am aware of how seemingly unpredictable—but also how fun—my own career path has been.

From Sea Urchins to the Human Retina

I was a postdoctoral fellow in the laboratory of Dr. Roger Pederson at the University of California, San Francisco, working with oocytes and the developing embryos of mice, when creation of the first transgenic mouse was reported (Brinster *et al.*, 1981). Brinster and colleagues had delivered a foreign gene into fertilized mouse eggs, transferred the eggs to foster (“surrogate”) mother mice, and then shown that the offspring not only expressed the foreign gene but passed it on to the next generation. These investigators then went on to create “super mice”—mice that grew twice the size of normal mice—by using the same technique, but substituting the gene encoding rat growth hormone for the thymidine kinase gene that they had used previously (Palmiter *et al.*, 1982). Instantly, the field of developmental biology was transformed and the possibilities for adaptation of this germline gene transfer turned science into science fiction.

The expansion of possibilities brought on by the growth of molecular biology was particularly exciting to me, because I realized that my studies in graduate school had been somewhat limited to the lack of molecular biologic techniques. In fact, I earned my PhD in zoology. Picture a desk with a van Leeuwenhoek microscope (as well as modern microscopes), open Erlenmeyer flasks containing liquid mercury on the shelves, and en route to the electron microscope you needed to step over some alligators. (Zoology departments at most institutions, including University of California, Berkeley, have since been restructured and renamed.) The atmosphere was enchanting, and I had totally enjoyed my graduate studies, which focused on the early development of sea urchin

embryos. My thesis mentor was Prof. Daniel Mazia, a distinguished biologist famous for his studies of the mitotic apparatus, the centrioles, microtubules, and other cellular structures responsible for mitosis. He allowed all of his students nearly complete independence, creating a sink-or-swim environment—which worked well for me and the majority of his students.

When I moved from graduate school to a postdoc, and from the echinoderm branch to the mammalian branch on the phylogenetic tree, I also moved from a university setting (graduate school) to a medical center (for my postdoctoral training). I naturally became interested in the prospects of focusing on research that could “help someone.” In Roger’s lab, I was really excited about the prospect of harnessing gene transfer to not only study mammalian development but also theoretically to identify clues to disease pathogenesis and hopefully ultimately deliver a treatment. Roger was a leader in mammalian developmental biology. His laboratory had the expertise for manipulating and transferring embryos to surrogate mouse mothers. What he needed was knowledge about the developing molecular techniques. When Roger asked me if I wanted to serve as the intermediary between his lab at University of California, San Francisco (UCSF), and a lab at the National Institutes of Health (NIH) in order to learn about transgenic mouse technology, I jumped at the opportunity. Roger made all of the arrangements, and I soon found myself commuting between Roger’s laboratory and that of Dr. W. French Anderson, a man who later became known as the “father of gene therapy.”

The experiences I had in French’s laboratory were pivotal with respect to my career and my life. This was my first exposure to molecular biology. I learned not only about cloning techniques and transgenic mouse technology but also about approaches being developed to deliver genes efficiently to somatic cells. French had already fine-tuned his vision of gene therapy by the early 1980s—the idea of developing treatments that would attack the source of the disease and that could potentially be completely curative. He was a clinician-scientist and was passionate about helping his

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patients. He was a high-energy individual—efficient, bright, and articulate—and also a fabulous mentor. His lab was huge, but he viewed it as his family. He was immensely proud of his wife, Dr. Kathryn Anderson, who had overcome substantial obstacles to become one of the first female pediatric surgeons in the country. French told me that because their careers were so full, the two of them had made the difficult decision not to have children so that they could dedicate their careers to helping others. Instead, French viewed each of his lab members as one of his “kids.” His office walls were covered from floor to ceiling with framed, professionally shot 8 × 10 black-and-white photos, each one of a different student, lab member, or lab alumnus. He was constantly being called for advice and letters of recommendation, and he enthusiastically helped each person.

French invited me, really just a visitor in his lab, to become involved by seeing the full picture of his research, including accompanying him to the clinic to see his patients. After working in the laboratory all day doing cloning, plasmid preps, and so on, it was especially motivating to see French with patients suffering from thalassemia, severe combined immune deficiency, and hypercholesterolemia. All of them were desperately seeking treatment and understood that he was working on it. Somehow, French also made time to carry on his outside activities—he was a martial arts teacher with a black belt in tae kwon do! In that realm, he also mentored hundreds of athletes. I wanted to do what he was doing (except that I didn’t want to eliminate the idea of having a family). On one of these trips to the clinic, I asked him for advice on developing my career as a “gene therapist.” He said, without any hesitation, “You should go to HMS [Harvard Medical School]. There, you will learn about the diseases that are in desperate need of treatments. And then you’ll know how to direct your research.”

It was simple. I would apply to Harvard Medical School (HMS), even though as an undergraduate a few years earlier, I had defiantly walked to all of my classes with a big button that said “I am not pre-med!” I went ahead and applied to that one medical school. I had decided that if I didn’t get into Harvard, I would continue my studies of developmental biology—perhaps applying molecular biologic techniques to sea urchin development and maybe even work at a marine biology station.

Somehow, I was accepted into HMS. After calling French to tell him and thank him for his advice, he jokingly said, “Now if you’re *really* lucky, the way I was, you’ll also fall in love with a fellow medical student.” I laughed, thinking that was absurd—that I would never date a fellow medical student. I next called Dan Mazia, who was really upset to hear the news that I was moving from research to the “dark side.” He was convinced that I would never get back to the bench. I promised him I would not give up on research. Roger Pederson was totally supportive and seemed to enjoy my prospects vicariously. I left for medical school, thinking maybe I could go back to UCSF at some point and work with him. As it turned out, Roger ended up leaving the United States for political reasons—he could not carry out studies with human embryonic stem cells in this country.

The field of medicine seemed to change dramatically over the time that I spent at HMS. As one example, when my class entered in 1982, gay men were succumbing to terrible infections, cancers, and deaths, but the disease didn’t even have

a name. The disease (HIV, AIDS) was identified by the time we graduated. In another example, new imaging technologies were being developed, and as usual medical students were invited to be the first guinea pigs. One of those was magnetic resonance imaging (then called nuclear magnetic resonance). And then there was gene therapy. I followed the progress attentively while I was in medical school and continued to do so while I carried out subsequent fellowships.

French’s lab (and others) fine-tuned retroviral expression vectors with selectable markers with the idea of introducing exogenous DNA into hematopoietic stem cells. French’s lab first used these vectors to deliver genes to mice. The plan was ultimately to deliver therapeutic genes to humans. French made his ideas clear to the public through a series of articles about scientific and ethical considerations addressed to both scientists and laymen. For example, he addressed the public’s uneasiness about somatic gene therapy, such as the fear that genetic engineering would be misused for purposes of enhancement. He also wrote about the regulatory process and the intense scrutiny inherent in the public reviews. At first, French’s essays were in a diverse set of publications. But soon, he had a regular column in the gene therapy journal that he cofounded in 1990 with Mary Ann Liebert, *Human Gene Therapy*. This was the first journal devoted to gene therapy and is, of course, the very one that has established the Pioneer Awards! *Human Gene Therapy* published the sometimes contentious public discussion—and the responses to this—of French’s plans for moving forward with the first approved human gene therapy protocol—one for a severe combined immunodeficiency (SCID) called adenosine deaminase (ADA) deficiency (Anderson *et al.*, 1990). ADA-SCID is a rare autosomal recessive metabolic disorder that results in severe compromise of the immune system. French went through 20 hrs of public hearings relevant to his proposed ADA-SCID studies, and these hearings were often hostile. Yet, he was determined to pave the way forward. And he did!

When the announcement was made that Drs. Anderson, Kenneth Culver, and Michael Blaese had accomplished the first approved gene therapy on a 4-year-old girl, Ashanti DeSilva (September 14, 1990), my husband, Albert M. Maguire, MD (who had been my HMS classmate and human brain-dissecting partner at HMS—I did, like French, end up finding my true love at HMS!), had a question relating to work he had been doing while embarking on a career in ophthalmology/vitreoretinal surgery: “Could gene therapy be used to treat retinitis pigmentosa?” My snap response was, “Yes, of course.”

However, I didn’t provide Albert with the long list of ingredients that would be necessary to develop a gene-based treatment for a blinding disease. In fact, none of those components existed at that time. (1) None of the genes involved in inherited blindness had been identified. Shortly thereafter (the year spanning 1989–1990), the first two genes involved in blinding diseases were identified (Cremers *et al.*, 1990; Dryja *et al.*, 1990; Farrar *et al.*, 1990). There are now at least 260 different blindness-related genes (RetNet, 2014). (2) There were no recombinant viruses identified that could deliver genes stably and safely to retinal cells. (3) There were no genetically characterized animal models of retinal diseases. (4) The surgical techniques for delivering genes to retinal cells had not been developed. (5) There were no metrics

described for measuring any effect of gene transfer on retinal/visual function.

My focus after medical school was to develop additional laboratory skills necessary to develop gene therapy. I was lucky that Albert's training took place in locations where there was exciting research relevant to gene therapy. I studied human genetics (ornithine transcarbamylase [OTC] deficiency) with Leon Rosenberg and Wayne Fenton at Yale and developmental genetics (focusing on Down's syndrome and Alzheimer's disease) with John Gearhart, Mary Lou Oster-Granite, and Roger Reeves at Johns Hopkins. Although I had been focusing on complicated systemic disorders, the idea of treating the eye intrigued me. My interest in the retina grew after collaborating with Drs. Donald Zack and Jeremy Nathans at Johns Hopkins on a project involving mice transgenic for a gene expressed in the retina (Zack *et al.*, 1991). The attractive features of the retina did not escape my attention; the retina is one of the few organs that can be examined noninvasively, the diseased cells take up a small amount of area (so that only a small amount of drug would be necessary), the eye has a favorable immune response with respect to foreign antigens, and retinal and visual function can be measured noninvasively. Further, there are two eyes, and retinal diseases are generally bilaterally symmetrical, so that one eye can be used as a control.

I took a gamble and wrote an application for a career development award from the then Retinitis Pigmentosa Foundation (now known as Foundation Fighting Blindness). The grant was awarded, and although this provided only a small amount of funding, it jump-started my career. These seed funds allowed Albert and me to develop the techniques with which to deliver (and assess expression of) recombinant DNA in the retina *in vivo*. We were also grateful to the Retina Associates at William Beaumont Hospital in Royal Oak, MI, who provided us with laboratory space while Albert carried out a fellowship in vitreoretinal surgery. The combined support likely played a part in our (Albert's and my) joint recruitment to the Department of Ophthalmology at University of Pennsylvania (Penn) in 1992, and also allowed me to collect enough data to submit a larger (NIH) grant proposal to develop gene therapy for retinal disease.

I was really excited about coming to the University of Pennsylvania for many reasons. One reason was that there was a large animal retinal disease study facility at the veterinary school. Sadly, one of the directors of this facility (Dr. Gustavo Aguirre) had just decided to leave Penn for Cornell, but fortunately, the other (Dr. Gregory Acland) planned to stay to run the facility. One of the most appealing features for me was the planned establishment of the Institute for Human Gene Therapy (IHGT), which opened in 1993. Dr. James Wilson had been recruited to direct this center by Dr. William Kelley, the dean of the medical school at that time. I had worked with Jim in the clinic when I was a medical student and he was a senior resident, but it wasn't until he was recruited to Penn that we actually talked about gene therapy. Jim and the IHGT recruited talented translational scientists from all different disciplines, established core facilities and a mission to educate scientists and laymen alike, sponsored seminar series and retreats, and provided expertise with which to help navigate the various translational hurdles. The enthusiasm for developing gene therapy was simply contagious. It was thanks to this facility

and Jim Wilson's enthusiasm for moving forward to develop ocular gene therapy that IHGT provided us with the first recombinant viruses (adenoviruses) with which to test the potential of somatic gene transfer to adult retinal cells *in vivo* (Bennett *et al.*, 1994). Similar studies were carried out simultaneously by Tiansen Li, then at Harvard (Li *et al.*, 1994).

We went on to demonstrate the first proof of concept of gene therapy-mediated intervention in a mouse model of an early onset form of autosomal recessive retinitis pigmentosa (ARRP) (Bennett *et al.*, 1996). Simultaneously, we carried out the first set of studies in the counterpart large animal model with Dr. Greg Acland (Maguire *et al.*, 1995). Although there was interest at IHGT in moving forward with recombinant adenovirus to test intervention of ARRP in humans, we and others soon recognized that another recombinant virus, adeno-associated virus (AAV), is much more efficient at targeting retinal cells than adenovirus (Bennett *et al.*, 1997). Further, AAV resulted in gene transfer lasting for years and had a favorable immunologic response (Bennett *et al.*, 1999, 2000). Our initial studies with AAV were carried out with help from Drs. Kris Fisher and Duangshen Dong, trainees at IHGT with Jim Wilson and John Engelhardt, respectively (with additional experiments with Drs. Vibha Anand and Fong-Qi Liang). We determined that AAV was equally efficient (and safe) at transducing photoreceptors of nonhuman primates (the best predictor for gene transfer effects in humans). We also tested the safety and transduction characteristics after readministration of AAV to the second eye of nonhuman primates. We found that to be effective and safe (Bennett *et al.*, 1999). This information paved the way for our studies leading to human clinical trials.

Unfortunately, the sad death of Jesse Gelsinger in the University of Pennsylvania-sponsored gene therapy trial for OTC deficiency that occurred that year (1999) put a huge damper on the field (Wade, 1999). All gene therapy trials came to a halt and the University of Pennsylvania IHGT was dismantled. With the belief that our approaches for treating retinal disease would be safe and effective, we continued to move forward cautiously with our bench research despite the unpopularity of gene therapy.

On July 25, 2000, we applied the procedures that we had developed over the preceding decade to test restoration of vision in a canine model of a severe, early onset progressive retinal degeneration called Leber's congenital amaurosis (LCA). Albert Maguire, Greg Acland, and I performed unilateral subretinal injections of AAV2 on three puppies born blind because of mutations in the *RPE65* gene. This gene had recently been discovered as the cause of a large fraction of cases of congenital blindness (Gu *et al.*, 1997; Marlhens *et al.*, 1997; Morimura *et al.*, 1998). The AAV (serotype 2) carried the wild-type canine *RPE65* cDNA and had been generated by Dr. William Hauswirth. The puppies had extremely poor vision and impaired retinal responses at baseline. Within a couple weeks after a single subretinal injection, each dog gained visual behavior and pupillary light reflexes. Later, psychophysical testing by Drs. S. Jacobson and A. Cideciyan (using electroretinograms) showed a restoration of retinal photoreceptor responses (Acland *et al.*, 2001).

There was discussion among our group of how to present the results to the press. Several collaborators wanted to avoid

mention of the animal model. I felt strongly that it was important to describe the impact of the treatment on this model because the implications would be obvious to laymen. I felt that the story would help stress the importance of biomedical research, show proof of concept of gene therapy with respect to blinding disease, and also provide hope not only for families suffering from a blinding condition but also for pet owners who care for blind animals. The story of one of the dogs, Lancelot, who also happened to be the most photogenic of the three, ended up being presented in the U.S. Congress and featured in media (including the television program *Good Morning America*, radio programs, newspapers, and even children's stories) around the world. In fact, a year later, I met with French Anderson at a gene therapy meeting, and he introduced me to a producer from the television program, *Animal Planet*, who also wanted to run a story. Lancelot settled for being named "Mutant of the Month" for the journal *Nature Genetics* (Bennett, 2003).

The obvious next thought was, wouldn't it be great to use this approach so that blind children could see? I filed an invention disclosure for this approach on behalf of all of the investigators involved in the canine LCA-RPE65 studies. Albert Maguire and I waived any potential financial gain on this intellectual property, which has since been awarded as a patent (Bennett *et al.*, 2012b). As a team, we planned to continue to work toward developing a clinical trial, and my lab members (including Drs. Nadine Dejneka, Tonia Rex, and Enrico Surace) helped develop the reagents and carry out experiments.

The team of investigators from the three universities (University of Pennsylvania, University of Florida, and Cornell University) was awarded a grant to run a phase 1 clinical trial for LCA-RPE65 from the NIH. Under this U10 grant, numerous preclinical safety and efficacy studies took place, including surgical procedures (all performed by Albert Maguire) and molecular, immunologic, histologic, and psychophysical studies (Acland *et al.*, 2001, 2005; Jacobson *et al.*, 2005, 2006a,b). At University of Pennsylvania, because of the tragedy of the Jesse Gelsinger case, the safety bar for human gene therapy protocols had been ratcheted up so that there were 13 committees that had to be navigated just to get to the point of submitting protocols to the local institutional review board (IRB) and then the Food and Drug Administration (FDA). Perhaps in part because of the regulatory burden, two of the investigators decided to move the studies to the Shands Hospital at the University of Florida. These investigators wanted to minimize the specter of Jesse Gelsinger and also be closer to the clinical vector storage facility, which was supported by the University of Florida-sponsored Applied Genetic Technologies Company. Instead of seeking privileges for Albert Maguire in Florida, a surgeon who had been taught the subretinal injection procedure by Dr. Maguire was recruited to carry out the surgery in adult patients at Shands Hospital.

The Exhilaration of Clinical Trials at The Children's Hospital of Philadelphia: The Power of Teamwork

When Dr. Katherine High knocked on my office door in July 2005 and asked if Albert and I would like to work with her to run a pediatric gene therapy clinical trial for LCA-RPE65, I went through a full spectrum of emotions—

excitement, disbelief, hope, joy, but beyond everything, a certainty that this would be the perfect opportunity to develop a safe gene therapy that could reverse blindness in children. This would complement the studies planned at Shands Hospital. Dr. High, a hematologist and gene therapy expert, had already been involved in clinical trials for factor IX deficiency (hemophilia B) (Kay *et al.*, 2000; High, 2004; Jiang *et al.*, 2006). She had been charged with establishing a Center for Cellular and Molecular Therapeutics at The Children's Hospital of Philadelphia (CHOP), the pediatric hospital affiliated with University of Pennsylvania. This hospital is routinely ranked "No. 1" in multiple areas in *U.S. News and World Reports* (<http://health.usnews.com/best-hospitals/area/pa/childrens-hospital-of-philadelphia-6231730/rankings>).

Kathy had hired individuals with the expertise necessary to carry out gene therapy translational studies, including experts in generation and purification of clinical-grade AAV (Dr. Frasier Wright), regulatory affairs (Jennifer Wellman), and in specific diseases (Dr. Valder Arruda). Additional talented individuals, including Junwei Sun, Dr. Federico Mingozzi, Dr. Shangzhen Zhao, Bernt Hauck, and Olga Zelenai, were recruited to help with the many additional requirements for carrying out the studies. As part of the mission, Dr. High was to establish gene therapy trials not only for hemophilia but also for diseases outside of her specialty. She knew about our experience and the published safety and efficacy data for LCA-RPE65 and felt that this would be a perfect fit for the goals of the pediatric hospital. We saw no conflict with our colleagues who had initiated operations in Florida aiming to treat adult patients. Further, there was no overlap in reagents or plans for recruitment.

We managed to move from the point of deciding to work on this trial through generating a clinical vector, building a full set of safety and efficacy data, breeding animals for study, designing clinical protocols and outcome measures, purchasing the necessary equipment, and enrolling the first subject in a short 2.3 years (Bennicelli *et al.*, 2008). Albert and I donated our time for all of these efforts, and Dr. Jeannette Bennicelli worked relentlessly with us on all of the laboratory studies until we were certain that everything was optimized. We had challenges identifying eligible subjects in the United States when we were ready to enroll, but thanks to our international collaborators (Drs. Francesca Simonelli, Alberto Auricchio, Enrico Surace, Sandro Banfi, Francesco Testa, Settimio Rossi in Naples, Italy, and Dr. Bart LeRoy in Gent, Belgium), we were able to move forward quickly. Additional subjects were recruited from the United States with help from Drs. Anne Fulton and Elias Traboulsi. We were also fortunate to have the expertise and dedication of clinical coordinator Kathleen Marshall, joined later by Sarah McCague and Dominique Cross. It was a bold step embarking on the first injection, although Albert's and my criteria for moving forward were far more stringent than any of the institutional or federal criteria. That was, if our child was affected with this condition, would we allow him/her to participate? Our definitive answer was yes!

Our phase 1 clinical trial at CHOP was carried out contemporaneously with trials at University College London and University of Florida Gainesville/University of Pennsylvania. It was the first to enroll pediatric subjects, a path that required a special review by the U.S. Recombinant DNA Advisory Committee (RAC) (2005). After a full review of the ethics of

enrolling pediatric individuals and presentations and cross-examination of our data and trial plans, the RAC voted unanimously to approve enrollment of these “vulnerable” subjects. The investigators running the other two trials were later able to leverage this approval. However, our study was the first to enroll children and to publish the full set of results. Our results demonstrated improved retinal and visual function in the treated eye of the majority of the subjects and were based on both subjective and objective findings (Maguire *et al.*, 2008, 2009). As we had predicted, the younger subjects, who had not suffered as much disease-related tissue degeneration, responded the best. All subjects showed significant gains in light sensitivity—a result that was also found by the University of Florida/University of Pennsylvania team (Cideciyan *et al.*, 2008). Our tests took advantage of traditional retinal and visual function tests as well as a mobility test, which we designed and implemented together with Dr. Daniel Chung (Maguire *et al.*, 2008, 2009). Interestingly, the University College London (UCL) team also used a mobility test (Bainbridge *et al.*, 2008).

Our team was also the first to carry out a follow-on study involving readministration to the contralateral eye. The readministration studies were carried out only after we had gone back to the laboratory to test the safety of this approach. The concern was that the initial injection of AAV would serve as a vaccination. Then, when the second eye was treated, an immune response might not only prevent benefit in the second eye but might also cause damage to the initially injected eye through immune sequelae. We tested readministration of high-dose AAV2.hRPE65v2 under conditions that we predicted would have resulted in a maximal inflammatory response. Fortunately, there were no harmful immune responses (Amado *et al.*, 2010). Albert and I adopted two of the affected dogs in that study, now sighted—our “seeing eye” dogs. To do so, we had to go all the way to the provost of the university to get permission. We joke that we now have our patients living with us!

We proceeded with the readministration studies in the human clinical trial subjects cautiously, enrolling the oldest individuals (least likely to benefit from the intervention) first and following them on a weekly basis. Again, the design of the studies benefited from the vast experience of the entire team. There was a 3-month stagger between enrollments of each of these individuals (Bennett *et al.*, 2012a). One was from a foreign country and thus could not travel back and forth each week. Also, she did not have a family member who could stay with her. Therefore, she lived with our family. This provided a unique opportunity to hear about the day-by-day improvements she noticed in her vision—and also an opportunity to improve our foreign language skills!

For the readministration studies, we added an additional outcome measure that was explored tangentially to the clinical trial—a study evaluating the restoration of visual responses in the brain (Bennett *et al.*, 2012a). The study was designed by Dr. Manzar Ashtari (Radiology, CHOP). Manzar had made functional magnetic resonance imaging (fMRI) measurements in our phase I subjects after they had already had their initial eye injected. There she demonstrated restoration of the neural circuitry connecting the retina and the visual cortex in individuals who had been deprived of vision for up to 35 years (Ashtari *et al.*, 2011). The subjects were able to see dim and low-contrast stimuli with their treated eyes—

targets that had been invisible to them before treatment. The areas of activation correlated closely with the areas of retina that had been treated. Data from the first three subjects enrolled in the readministration study have been reported (Bennett *et al.*, 2012a). The results represent the first successful human gene therapy readministration. The remaining eligible subjects have been enrolled and injected, and there have been no severe adverse events related to the vector. The long-term follow-up data will soon be available. Besides the fact that there is benefit from injecting the second eye and that the readministration was safe, the data are really exciting with respect to getting a handle on the nature and limits of plasticity of the central nervous system (using fMRI). It had previously been thought that the brain would not be able to interpret signal delivered from the retina if retinal function was restored beyond the “critical period” of vision development (i.e., greater than ~3 years old in humans).

The phase I/II studies have been extraordinarily exciting and personally rewarding. The team effort was incredible, with each person doing whatever it took to make the study safe and informative. I truly believe that the team is key with respect to success! Few scientists have the opportunity to see a study move from the bench to the clinic, and this is a process that requires a tremendous amount of complementary expertise as well as the ability to work well together.

Our next challenge was to make a plan on moving forward for a pivotal (phase 3) study—one that would potentially allow the reagent to be labeled as a drug and to be prescribed for individuals who might be able to benefit. There has been no precedent for designing such a study. Further, we were given certain constraints by the FDA, for example, the need to have an untreated control group in order to show that symptoms of this condition do not spontaneously improve over time. We knew that if we had a control arm where individuals had no chance of intervention, that few people would enroll, because most individuals had heard about the improvements gained by the treated subjects. We came up with a plan, however, to randomize subjects 2:1 intervention:control, but to have the control subjects cross over to the intervention arm after 1 year (Spark Therapeutics, 2014).

That also partially satisfied a need for natural history data. The phase 3 study is well underway and all of the subjects have been enrolled. Barring any unplanned events, we expect the first data set to be ready for submission to the regulatory agencies in the last quarter of 2015. If this reagent is indeed ultimately approved for human use, it could be a huge step for the gene therapy world. This could be the first gene therapy product approved in the United States and the first ocular gene therapy product approved worldwide. The data could pave the way for other ocular gene therapy products. There are now more than a dozen ocular gene therapy trials underway, and another dozen or so targets are being considered by various groups. This bodes well for developing gene-based treatments for blinding diseases.

Gene Therapy Will Shed Light on Biology

True to the reiterative nature of gene therapy development and transfer of hypotheses back and forth between the laboratory and the clinic, biologic questions will remain even after the first retinal gene therapy drug is approved. Additionally, there will likely be reiterations that further

improve the efficacy. Will gene therapy treatment prevent further degeneration of the retina if it is applied early in the course of the disease? At this point, we do not know, although one group has used unconventional modeling in a small number of human samples to suggest that the answer is no (Cideciyan *et al.*, 2013). We feel that this question cannot be answered as of yet, and also that the answer will depend on a number of variables. In the meantime, gene therapy is the most promising treatment at hand. Even if it lasts only as long as it did in Lancelot (11 years), the effects would be extraordinarily meaningful to the patients (Wojno *et al.*, 2013). Other questions are: Will it be possible to safely readminister gene therapy to the initially injected eye? Are there exercises that might help persons to nurture their newly acquired visual abilities postinjection? What are the limitations with respect to plasticity of the brain with respect to understanding new vision delivered via the treated retina? We still have a lot to learn!

What Next?

How difficult will it be to implement retinal gene therapy across the United States? The surgical procedure is thought to be something within reach of any board-certified vitreoretinal surgeon, yet like any procedure, there is a learning curve. It will thus be important to develop a training plan so that the drug can be administered by “certified” surgeons. It will also be important for all of the potential patients to receive clinical laboratory-approved molecular diagnoses. This is not always covered by insurance and so this step will impose some economic challenges.

What are the next disease targets? There are plenty of them, including disease involving cilia structure/function. We are tackling some of these (Boldt *et al.*, 2011; Drivas *et al.*, 2013) (which would have made my now-deceased PhD thesis advisor, Dan Mazia, happy!). One question is how to move more quickly from proof of concept to planning of a critical trial? It may be possible to streamline this process by carrying out some of the proof-of-concept studies in personalized *in vitro* models instead of in animal models. We have tested this approach in moving forward to develop a gene therapy clinical trial for choroideremia (Vasireddy *et al.*, 2013). So far, the FDA has not provided resistance to our *in vitro* approach.

What Are the Obstacles for Developing Gene Therapy for Other Retinal Applications?

First, how does one select a viable gene therapy target? There are many variables that affect the ranking of disease targets. These include both scientific and nonscientific issues ranging from funding, intellectual property, immunologic issues, numbers of patients with molecular diagnoses, the level and cellular specificity of transgene expression that is appropriate, the selection of outcome measures, and regulatory hurdles. Economic issues may be the most challenging. It costs an enormous amount of money to run a clinical trial. It could easily cost millions of dollars to run a phase I clinical trial. Plus, it is mandatory to follow subjects in gene therapy clinical trials for 15 years, even if the primary endpoints are only studied for 1 year.

Will it be possible on an economic basis alone to develop gene therapy for each of the 260-plus different inherited

retinal diseases? Maybe over time, it will become possible to consolidate preclinical data sets, thereby reducing time and expenses by addressing safety concerns inherent to a new transgene *in silico*. Former mentee Luk Vandenberghe has some creative ideas relevant to this approach. Alternatively, perhaps the need to run a good laboratory practices (GLP) toxicity study for each molecular form of blindness can be waived, as it is orders of magnitude cheaper to carry out such studies under standard (academic) laboratory conditions. At present, even a small change in a vector automatically labels it a “new” vector, thereby requiring a formal GLP toxicity study.

Aside from global financial aspects, the pressures on academic investigators (who take care of most orphan disease patients) are intense. The need of the hospital to stay financially intact affects the workload of doctors and support for clinical studies. By spending time running a clinical study, the physician is not doing cases that bring in money to the institution. The regulatory responsibilities of a gene therapy clinical trial are significant. Add to that the fact that a principal investigator takes responsibility for any complication or adverse event that may occur, and few doctors are willing to take on the task. There are certainly no financial incentives for participating in a clinical trial as (at least at University of Pennsylvania) an investigator cannot hold any equity/financial interest in a product that he/she is investigating (or a spouse is investigating).

Both Albert and I waived any potential benefit so that he (and I) could serve on the study team. We knew that it would be an intense and exhausting process to develop and run a gene therapy clinical trial especially with a short timeline, and we were ready for the commitment. We would not have done anything differently, but it will be difficult to find other individuals who are willing to work as hard as we did, and without compensation. There were plenty of times when we felt that we could not tolerate one more regulatory roadblock. All it took each time, however, was to see firsthand the incredible impact of the intervention—for example, to see a child who had previously been navigating with a blind cane, catching fireflies and playing hide-and-seek, to know that a mother can now see her child’s face, or to see a college student newly able to engage in social and athletic events instead of standing by the wall—to renew our energies.

A much broader issue deals with the future cost of an approved drug to the consumer. Will the approved gene therapy drugs be provided by nonprofit academic centers, or will they be provided at high cost by industry? If the trend of the past year in the increase in newly established gene therapy companies interested in ophthalmic disease continues, it is likely that industry will generate the majority of the products. Will health insurance cover the costs of the drug? If so, what are the limitations? Will people who do not have insurance be eligible? If they need the drug, will they have to mortgage their homes? If the drug is effective for only 5 years (instead of 10), will there be a rebate? Will there be compassionate use of certain drugs and, if so, who will pay for that?

Compassion

The entire field of gene therapy was built with compassion and the goal that human suffering can be ameliorated or even prevented. Tragically, W. French Anderson, the father

of gene therapy, and the very person who encouraged and motivated me to enter this promising field, is languishing in prison, where he has already spent 8 years after being prosecuted for criminal charges he tells his story on a website with his name. Besides the devastating consequences on his life, his wife's life, and other lives, this has been a huge blow to the field of gene therapy. I truly hope that French can be released soon so that he can focus the remainder of his life on the good that has evolved from his passions for translational medicine and sports. It would be wonderful for him to see his decades-old predictions about the power of gene therapy materialize. I hope that the countless numbers of scientists, athletes, and patients whose lives he has so wonderfully affected will be able to give him their personal thanks.

In summary, I am very grateful for the unique opportunities that I have had, and the many talented individuals I have had the fortune to meet. Together with my husband, I have been very fortunate to be able to recognize and harness the opportunities and witness the successful translation of our laboratory work into the clinic. This has been extraordinarily fulfilling. Throughout my career, I have been mindful of the impact a mentor can have on a young physician/scientist and of the importance of ethical behavior and respect for trainees, collaborators, colleagues, and team members. I hope that my story will help to remind all scientists of the positive impact they can have on successive generations of scientists by sharing their enthusiasm and being both good role models and mentors.

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Author Disclosure Statement

J.B. is a scientific advisor for Spark Therapeutics, Avalanche Technologies, and Novartis; a founder of GenSight Biologics; and a member of a monitoring committee for Sanofi.

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