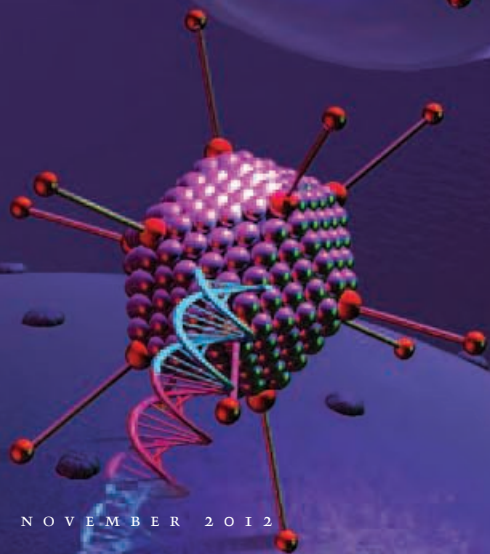


ADVANCES IN

Retinal



From inherited retinal dystrophies to AMD, the pace of gene therapy is picking up, spurred on by recent success with Leber congenital amaurosis. An update on current research and insights from leaders in the field.

Gene Therapy

BY ANNIE STUART, CONTRIBUTING WRITER

After a few false starts in early gene therapy clinical trials in the 1990s, the dramatic success of the Leber congenital amaurosis (LCA) trials has spurred renewed interest and a great deal of development in the field at large. Although research is progressing in uveitis, glaucoma, and cornea, the most promising results in ophthalmology thus far have emerged with retinal disorders.

As with so many areas of study, the eye offers a unique opportunity for gene therapy. “Because of its size, the eye requires relatively small doses to achieve a therapeutic effect,” said J. Timothy Stout, MD, PhD, MBA, genetic researcher and professor of ophthalmology at Oregon Health & Science University in Portland. This was particularly advantageous at the very earliest stages of eye gene therapy, he said, when making large amounts of gene vectors was no easy task. Small doses and localized treatment also translate into lower risks of systemic toxicity. And the contralateral eye provides a convenient control.¹

The eye is also anatomically isolated, immune privileged, and easily accessible. By surveying its landscape with high-resolution imaging, genetic researchers can see the results of the viral vector injections noninvasively and within a matter of minutes, said Dr. Stout.

That’s a far cry from evaluating gene therapy for liver disease, for example, where it’s not possible to make direct observations.

Retinal Rewards

The retina is a desirable target for gene therapy, largely because it is an essential, irreplaceable part of the central nervous system, said Richard A. Lewis, MD, professor of ophthalmology and molecular and human genetics at Baylor College of Medicine in Houston. “You can change some things about the anterior segment of the eye—repair corneal damage, do transplants, or remove cataracts—but you can’t replace the retina.”

From inherited retinal dystrophies to AMD, gene therapy offers promise for the clinician in two primary ways: “There are a number of retinal diseases for which there are zero therapeutic options,” Dr. Stout said, “and a number for which we have therapeutic options, but the delivery of the therapies is either ineffective or cumbersome.” For example, he said, sustained genetic expression in the retina might obviate the need for repeated treatments such as injections of biologics for AMD, thereby lessening the risks of local side effects including infection or retinal detachment.

Vector Variables and Surgical Scenarios

Manipulated to become virtually benign, viral vectors are the space shuttles of gene therapy. With a range of cargo capacity, stability of expression, immunogenicity, and differential targeting of cells, three main types of vector have been used for retinal gene therapy: adeno-associated virus (AAV), lentivirus, and adenovirus (AV).¹

Tailored usage. “As with a toolbox of antibiotics, we use different vectors for different purposes,” said Dr. Stout, reflecting on the tremendous progress made since his graduate-school days, when he and colleagues were first tackling a prerequisite to therapeutic gene delivery—figuring out how to make viruses capable of transferring genetic information into cells.

Today, however, the world of vectorology is accelerating quickly, said Jean Bennett, MD, PhD, professor of ophthalmology at the University of Pennsylvania in Philadelphia and scientific director for phase 1/2 human LCA clinical trials. “People are developing multiple serotypes—‘flavors’ of the reagent—with different attributes, some of which may be beneficial for particular diseases,” she said. However, each new vector requires detailed safety studies to determine whether it goes outside a target area, attaches to the wrong cell type(s), or induces an immune response, for example.

AAV vectors. Used in LCA trials, AAV vectors have proved to be remarkably safe, said Dr. Stout, and they can express the contained genes for long periods of time. “We think that patients we’re treating for LCA may well be cured, because they’re going to be making RPE65 protein for the long haul.”

AAV vectors transduce cells efficiently, added James W. Bainbridge, MA, PhD, FRCOphth, professor of retinal studies at the University College London Institute of Ophthalmology. “Now there is a wide range of serotypes that can allow a range of cell targeting,” he said, adding that one disadvantage

of AAV is its relatively small capacity, which can pose a problem with larger genes, such as the gene involved in Stargardt disease.

Lentiviral vectors. By contrast, said Dr. Bainbridge, large carrying capacity is an advantage of lentiviral vectors.

Although these vectors transduce retinal pigment epithelium (RPE) cells very efficiently, they are less efficient than AAV vectors at transducing photoreceptor cells.

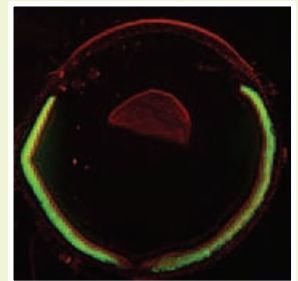
Unlike AAVs (and AVs, below), which are basically inert delivery systems, lentiviruses function by incorporating themselves into cells, said Dr. Lewis. However, no gene therapy trial with a retrovirus has ever gone forward without proof that the virus is replication negative, said Stephen M. Rose, PhD, chief research officer for the Foundation Fighting Blindness in Columbia, Md.

AV vectors. Used more commonly in the past, AV vectors elicit expression for only about 90 days, said Dr. Stout. “However, there might be clinical scenarios where turning off or modulating something for a brief period of time might be just fine.”

Nonviral delivery. Although they aren’t quite ready for prime time, nonviral vectors carry great promise, said Dr. Bennett. Whereas lentiviruses top out at 8,000 or 9,000 base pairs, these lipid- and nanoparticle-based vectors have no limitation in packaging capacity, she said. “Most use a trick to compact the DNA or RNA to allow it to transfer across the lipid bilayer of cell membranes,” she said. They also don’t carry viral proteins with the potential to induce an immune response, she added. However, they may lack the staying power of some viral vectors.²

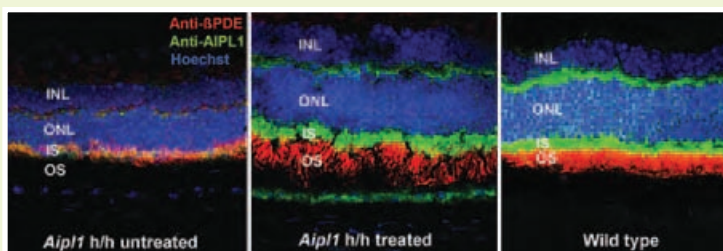
Surgical approaches. Given the vectors currently in use, retinal gene delivery now involves a standard vitrectomy and subretinal injections, said Dr. Stout. “But that may not be the way we deliver therapeutic material forever. People are working on modifying the capsid proteins of the AAV,” said Dr. Stout, which may allow it to transduce the same cells using intravitreal injections.

Additionally, researchers are investigating less invasive surgical approaches, such as gaining access to the retina and RPE through the suprachoroidal space. This involves making a small cut in the sclera and threading a cannula along the wall of the eye to the back of the eye. “We have good data to suggest we’ve obtained transduction of the photoreceptors



Cross-section of a mouse eye following subretinal injection of AAV carrying the green fluorescent protein gene. Efficient gene delivery can be achieved, targeting almost 100 percent of the photoreceptor and RPE cells.

Gene delivery using the AAV 2/8 vector restores expression of AIPL1, a protein missing in a model of severe early-onset retinal degeneration.



and the blood vessels,” said Dr. Stout.³ Such less-invasive approaches, which preserve the integrity of the retina, are particularly important in certain circumstances, said Dr. Rose. “In cases like retinoschisis, where your retina looks like Swiss cheese, you really don’t want to disturb what’s there.”

LCA Lessons—and a Caution

Beginning in 2008, independent studies of patients affected with LCA due to mutations in the *RPE65* gene were published by researchers in London, Philadelphia, and Florida. At the time, it was known that the RPE65 protein played a “recycling role” in the visual pathway and that a subset of LCA patients did not make this protein. However, it wasn’t known whether the simple act of supplying a patient with a normal copy of the gene would slow or reverse the disease. “Getting normal copies into enough RPE cells slowed down degeneration in the dog model, and, as it turns out, this works remarkably in humans,” Dr. Lewis said.

“The LCA studies demonstrated proof of principle for a technique that could be widely applicable to a number of inherited retinal conditions, particularly those in which a single gene is responsible for lack of function of an essential protein in the retina,” said Dr. Bainbridge.

Safety. “The primary message from the LCA trials is that gene transfer to retinal cells is safe,” said Dr. Bennett, “at least at the doses and volumes tested in the particular AAV vector we used.”

Using a similar AAV vector, Dr. Bainbridge and his colleagues chose to target expression of the gene specific to the RPE cells through use of an *RPE65* promoter, which minimized inappropriate expression of *RPE65* in cells other than the RPE. “We felt this added a degree of safety, which was our prime concern,” he said. Determining safety was particularly important, said Dr. Bennett, as the AAV vector could potentially be used to treat many other blinding conditions. (It has, in fact, already gone into the clinic for other indications.)

Follow-on LCA studies are nearing completion, said Dr. Bennett. They involve treating the fellow eye in up to 11 patients who previously received an injection in one eye. Safety is still a major focus. “Our concern was that the initial injection of the reagent acted like a vaccination,” she said. Would a second injection of the same material trigger inflammation, preventing benefit to the second eye or worsening vision in the first? Fortunately, these concerns have not materialized, either in animal models or in humans, she said.

Efficacy. “The ETDRS acuity test often confirms better sight in the treated eye,” said Dr. Stout, “but at the end of the day, it’s about the patients and how

it affects their vision. We’ve got some kids who clearly see better in the treated than in the untreated eye,” he added, describing children who can now ride bikes or navigate better in the dark, thanks to improved photoreceptor function. In others, their nystagmus has resolved.

Further studies have shown additional benefits of treating the second eye, said Dr. Bennett. However, she noted that LCA trials have highlighted the need for more clearly defined outcome measures, beyond eye charts and visual fields, for patients receiving gene therapy. “These tests either are not useful or have limited utility for many diseases. There are other features of vision that are clinically meaningful, such as the ability to see in dim light or to navigate independently.”

Timing. The LCA trials appear to show that the earlier the treatment, the better the outcome, said Dr. Stout, who added that this may not hold for all diseases and for all forms of gene therapy. Even so, patients who have few cells left may still benefit, said Dr. Bennett. “It may not seem significant to someone with 20/20 vision, but the meaning is huge for someone who couldn’t see a face and can now tell whether a person is smiling or frowning.”

Question of endurance. Dr. Lewis offered a caution: “The third critical goal, after ‘safe’ and ‘effective,’ is ‘durable.’ No matter how safe and no matter how effective any gene-replacement or biological therapy may be, if it lasts only a week or two and must be repeated, it may not be useful for a lifelong and degenerative disorder of the retina.” It’s too soon to tell whether this goal will be reached.

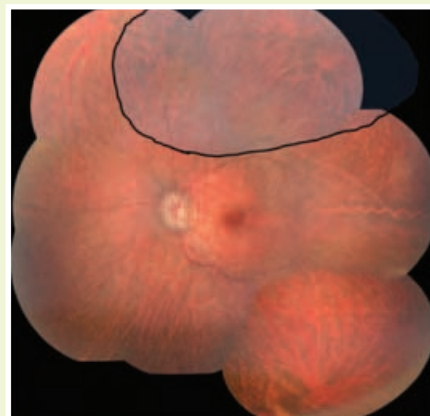
Beyond LCA: New Horizons

Although retinal gene therapy research began with monogenic retinal diseases, retinal gene researchers also have their sights set on myriad other diseases.

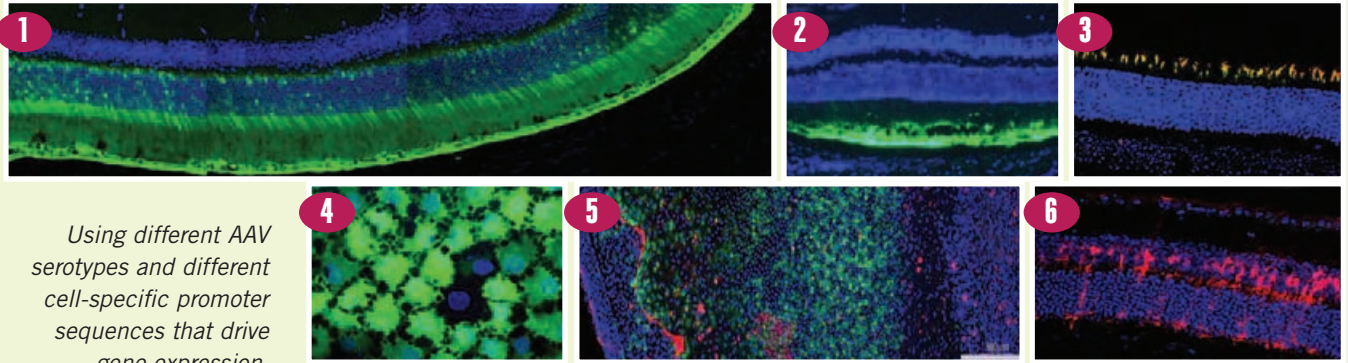
At present, nine clinical trials are under way for inherited rare retinal dystrophies, and between 20 and 30 are evaluating gene therapy for wet and dry AMD, said Dr. Rose. In addition, “old-fashioned natural history studies” are helping to reveal the impact a gene variant has on the progression of a particular disease. “We’re just beginning to understand the personalized portion of these diseases and variants.”

Strategies include the following.

Modulating pathways. In some cases, replacing a



Extramacular subretinal bleb (shaded area) in patient with LCA.



Using different AAV serotypes and different cell-specific promoter sequences that drive gene expression, gene delivery can be targeted to (1) photoreceptor cells and RPE, (2) RPE only, (3) cone photoreceptor cells only, (4 and 5) corneal cells, and (6) Müller cells in the inner retina.

gene can restore sick cells or preserve cells that are alive or threatened, stopping or even reversing the disease process, said Dr. Stout. “But many of the pathways that involve disease don’t necessarily occur because of a defective gene.” It might be an otherwise normal gene being expressed inappropriately in a temporal or spatial way. In these cases, gene therapy can be used to modulate a pathway, for example, preventing blood vessel growth or turning down the immune system.

Overcoming lack of function. Much of the focus of current retinal gene therapy, said Dr. Bennett, is on mutations that prevent a protein from forming, resulting in so-called lack-of-function diseases. In LCA, for instance, the mutations in the *RPE65* gene prevent formation of an enzyme involved in the

vitamin A cycle. The data show that by delivering a normal copy of the gene, you can overcome the biochemical deficit, she said.

“I think the same thing will be true in other diseases caused by a lack-of-function mutation,” said Dr. Bennett, pointing to gene therapy trials recently initiated for Stargardt disease, Usher syndrome, and choroideremia. Preliminary results indicate the approach is safe, and early signs of efficacy are evident, Dr. Bennett said.

Intervening in early-onset disease. Dr. Bainbridge has a particular interest in working with early-onset conditions that cause severe, predictable visual loss or severe lack of function, such as achromatopsia. “These conditions provide the opportunity to measure the impact of treatment in a relatively short time period,” he said. “Less severe retinal degenerations can progress relatively slowly over a period of years

IS THERE LIGHT AFTER DARK?

No treatment yet exists to restore vision after rods and cones have been lost and are no longer sending signals to the brain. But the novel field of optogenetics—the combination of optics and genetics—may one day bring more than a glimmer of hope to those with degenerating retinas from diseases such as RP and AMD.

Essentially, optogenetics involves maximizing the use of remaining cells by stimulating the visual transduction pathways with light-sensing molecules from bacteria or algae.¹

“It’s another area with a lot of potential, especially in scenarios where there may be a variety of different genes that might be defective,” said Dr. Stout, “or where we can’t put a gene into the appropriate cells in the right way.”

When some cells are left. Two optoge-

netic approaches are being supported by the Foundation Fighting Blindness, said Dr. Rose. One involves putting light-sensing molecules (halorhodopsin) into the remaining inner segment of photoreceptors before they die. This protein, which is similar to photopigments in human photoreceptors, allows remaining photoreceptors to do what they’re capable of doing, which is to take the electrical signal, feed it down through the trunk line, and take it back to the brain, said Dr. Rose. “Will these cells perceive light and will this stop the degeneration? From early studies, the answer appears to be yes.”

When no photoreceptors are left. But what if the “building” is demolished down to the foundation? Can the retinal ganglion cells, which are essentially the signal-transmitting “electrical lines” in

the building foundation, still carry a signal back to the brain? Preliminary results appear positive. With genetic engineering that turns the retinal ganglion cells into light-sensing cells by adding channelrhodopsin-2 from green algae, these cells become both signal gatherers and transmitters.

“I’m cautiously optimistic,” said Dr. Rose. “There is evidence that mice with blinding photoreceptor degeneration will gravitate toward light after this treatment with optogenetics. But we don’t have any clue about acuity.”

The data are compelling, agreed Dr. Bennett. “Next steps will be to see whether it is effective and safe in a large animal model, and then in humans.”

1 Cronin T, Bennett J. *Mol Ther.* 2011;19(7):1190-1192.

and, sometimes, decades, so these are more likely to require prolonged, expensive studies to determine the impact of the intervention.”

Retinitis pigmentosa (RP), which is caused by an X-linked mutation in the GTPase regulator (*RPGR*) gene, is another early-onset, rapidly progressing disease. Although the gene posed challenges for the investigators, said Dr. Bennett, recent preclinical trials at the University of Pennsylvania have shown some evidence of efficacy in three dogs, perhaps laying the groundwork for future studies in humans.⁴

Reversing double trouble. In some cases, said Dr. Bennett, a mutation alters a protein and makes it toxic, doubling the challenge. “You have to think about how to remove that toxic gene as well as introduce a normal copy. But there is beautiful proof-of-concept data in animal models,” she said, pointing to autosomal dominant RP studies looking at particular rhodopsin mutations, “and I think it’s only a matter of time before it’s tested in humans.”

The suppression and replacement gene therapy, which involves subretinal administration of two AAV vectors, corrects the genetic defect in a mutation-independent manner.

Keeping cells alive. The ability to deliver genes to the retina provides an opportunity to establish sustained expression of therapeutic proteins inside the eye for a number of different complex conditions, said Dr. Bainbridge. “But with more than 200 genes identified in inherited retinal degenerations, you’re certainly not going to do 200 separate gene therapies,” said Dr. Rose. “Instead of correcting a primary defect, you might provide a signal and a growth factor for cells to help keep them alive.”

Generic gene delivery strategies such as this use of neurotrophic factors are of great interest, added Dr. Bennett, because they hold the potential to help many more people than does single-gene therapy.

Conquering dividing cells. Another generic approach is to target cells with a vector that would allow the transduced cells to be killed. This tactic might inhibit the scarring processes that cause retinal detachments or neoplastic diseases such as melanoma or retinoblastoma, said Dr. Stout. For instance, in AMD, expression of the VEGF gene causes blood vessels to grow or at least supports their growth. “There’s the possibility that we could use viral vectors to transduce cells to produce and secrete antiangiogenic factors for the life of the patient,” he said.

In experimental models, Dr. Bainbridge’s lab has used viral vectors to deliver inhibitors to the retina and has demonstrated sustained expression and control of neovascular forces, including choroidal neovascularization.⁵ Two clinical trials modeled after this approach are currently in progress. Results will be closely watched, said Dr. Bennett, given that neo-

vascular disease occurs in many forms, from diabetic retinopathy to corneal neovascularization.

Time Is of the Essence

In reflecting on the impetus gained from the LCA clinical trials, Dr. Bennett summed up the challenge for herself and other genetic researchers: “What is getting me up in the morning and keeping me up really late is trying to extrapolate lessons to the next disease. I’m driven to do that because there’s a window of opportunity. People see their vision disappearing, and I feel like it’s our responsibility to make this happen soon so they have a chance.”

1 Liu MM et al. *Br J Ophthalmol*. 2011;95(5):604-612.

2 Conley SM, Naash MI. *Prog Retin Eye Res*. 2010;29(5):376-397.

3 Stout JT, Francis PJ. *Hum Gene Ther*. 2011;22(5):531-535.

4 Beltran WA et al. *Proc Natl Acad Sci USA*. 2012;109(6):2132-2137.

5 Bainbridge JW et al. *Gene Ther*. 2002;9(5):320-326.

MEET THE EXPERTS



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