GENE THERAPY AND RETINAL DISEASE

Gene replacement therapy emerging as a novel approach in the treatment of many retinal disorders once deemed incurable

by Dermot McGrath

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Gearóid Tuohy

Gene therapy will be a significant contributor to the future armamentarium of therapies for at least some of the hereditary blindness conditions that are currently incurable

Artur V Cideciyan PhD

It has been estimated that one in every 4,000 people suffers from an inherited retinal disease and half of those affected have no family history of the pathology. Individuals diagnosed with diseases such as Leber’s congenital amaurosis (LCA), choroideremia, Stargardt’s disease, Usher’s syndrome, certain forms of retinitis pigmentosa (RP) as well as other inherited dystrophies typically have a poor visual prognosis and treatment options are currently limited.

In recent years gene replacement therapy has emerged as a novel approach in the treatment of many retinal disorders once deemed incurable. The principle of gene therapy is based on the transfer of a therapeutic gene or part of a gene administered to the eye by use of viral or non-viral vectors.

After a succession of early clinical failures led many to dismiss the technology as over-hyped, that view has been revised due to notable breakthroughs in gene therapy treatments over the past five years.

In the field of ophthalmology there is real hope that the eye can showcase the potential of gene therapy to delay, arrest, reverse or ultimately “cure” a range of blinding diseases.

As geneticists have long since noted, the eye is a particularly amenable target for gene therapy. It is one of the few immunologically privileged sites in the body and is therefore less likely to provoke a systemic immune response from gene vectors injected subretinally.

Moreover, the defined volume of the eye means that minute amounts of viral vectors may be all that are necessary to achieve localised therapeutic effects without the risk of systemic toxicity and absorption that comes with intravenous drug delivery.

Furthermore, major advances in ocular imaging and the localised nature of retinal pathologies means that treatment effects can be more readily observed and monitored for efficacy and safety than with systemic conditions.

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Dr Tuohy stressed the significance of Dutch biotech company uniQure’s achievement in developing the first gene therapy product – which is radically changing perceptions of the field.

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Dr Tuohy stressed the significance of Dutch biotech company uniQure’s achievement in developing the first gene therapy product to be approved by the European Medicines Agency (EMA) in July 2012. Its product, Glybera® (alipogene tiparvovec), is designed for the treatment of lipoprotein lipase deficiency (LPL), a rare autosomal recessive disorder affecting about one in a million people.
Early results from first choroideremia trials

Other positive news has been emerging from the first clinical trial in humans of a gene therapy for choroideremia, an inherited retinal degenerative disease caused by a defective Rab escort protein-1 (REP1) gene. The disease is X-linked recessive and leads to the degeneration of the choiopapillaris, the retinal pigment epithelium and the photoreceptors of the eye.

Starting in October 2011, a team at John Radcliffe Hospital in Oxford led by Prof Robert E MacLaren of the University of Oxford began treatment of the first of six patients using a modified AAV vector to deliver the replacement gene.

With the study outcomes currently under review prior to publication, Prof MacLaren told EuroTimes that the six-month data look very promising.

“What we discovered was that most choroideremia patients have subtle defects in retinal function that are similar in some ways to a mild form of LCA. It is not obvious at first because most can read 6/6 or better on a Snellen chart, but it is easily seen on microperimetry. This is helpful for a gene therapy trial because improvements in visual function serve as an early indicator of successful gene transfer. Of course we do not know early on if the degeneration has stopped, but with an improvement in vision we can at least assume that the transgene has been activated in the cells. The first six patients have now reached the full six-month follow-up and we will be reporting our results in full shortly, however, we can say that there have been no serious adverse events in any of these patients and the gene therapy appears to be very well tolerated,” he said.

“What is particularly exciting about this trial is that we can also see for the first time the effects of AAV gene therapy in patients who have a near normal foveal

Possible solutions exist, however, to tackle the problem of continued neurodegeneration regardless of the successful reactivation of rods and cones in treated areas.

“This could potentially be addressed by a combination treatment of a vector containing the specific gene for correcting the disease, for example RPE65, and a second viral vector or encapsulated cells containing complementary DNAs for neuroprotective or anti-apoptotic proteins,” said Dr Stieger.

“Such an approach in non-specific gene therapy has been tested in many animal models and also with the use of ciliary neurotrophic factor in encapsulated holofibres to protect neural cells, including photoreceptor cells, in human patients as developed by Neurotech in the United States,” he said.

While promising techniques do exist for gene therapy augmentation with adjuvant treatments, a lot more basic research is needed to assess their safety, cautions Dr Cideciyan.

“Such treatments could involve induction of cell-protective pathways or inhibition of cell-death pathways with the use of neurotrophic factors, antioxidants and apoptotic inhibitors. Pre-clinical studies using such approaches will have to demonstrate proof-of-concept and safety before clinical trials can be planned,” he said.

Tackling retinal degeneration

While the latest findings are undoubtedly a setback, clinicians are already probing possible methods to overcome the issue of ongoing retinal degeneration after successful gene therapy.

“We know now that gene expression following AAV mediated gene transfer is efficient and safe. So the technology has reached a point where substantial cure is possible if intervention takes place in tissue that still contains photoreceptors and RPE cells to be cured,” said Knut Stieger DVM, PhD, of the University Eye Clinic in Giessen, Germany.

“This point cannot be stressed enough – AAV-mediated gene addition therapy only works if there are target cells remaining. Therefore, identification of patients, or more strictly speaking, treatable retinal tissue by OCT and functional tests is the key to successful gene therapy,” he added.

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NGS underpins gene therapy progress

Deciphering DNA sequences is essential for virtually all branches of biological research and ophthalmology is no exception. In recent years, the advent of next-generation sequencing (NGS) has transformed gene therapy research, enabling rapid sequencing of large stretches of DNA base pairs spanning entire genomes, with the latest instruments capable of producing hundreds of gigabases of data in a single sequencing run.

Retinal diseases are prime candidates for NGS because of their extensive genetic heterogeneity, explains Prof Hanno J Bolz, deputy medical director at the Biocentrum Cologne. His group has identified several novel genes for Usher’s syndrome, RP, Joubert’s syndrome, ataxia and deafness.

NGS also assists in rapidly identifying the remaining unknown disease genes, notes Prof Bolz. “Targeted NGS of panels comprising all known genes for a disease group such as LCA or RP are an excellent first step. In research, those samples that remain mutation-negative in this test are excellent candidates for exome sequencing, enriching and sequencing virtually all exons of all human genes,” he said.

Obtaining data, however, is only a small part of the picture, points out Prof Bolz. “Generating the data has become less of a problem but interpretation is now crucial. For macular dystrophies, for instance, one has to differentiate between the monogenic forms of macular disease and choroidal retinal dystrophies, where a single mutation in one gene is sufficient to cause the disease, and polygenic forms like AMD where there are variants rather than mutations that predispose to disease, but not necessarily evoke it in all carriers.

The pace of progress in gene sequencing in recent years has been phenomenal, said Prof Bolz.

In each case, and especially in a diagnostic setting, one has to make sure that interpretation of next-generation sequencing data is sound and to the benefit of the patient.

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“What we do now is second-generation sequencing but third-generation sequencing which denotes single-molecule or nanopore sequencing is already available but needs to be refined in terms of accuracy and cost effectiveness. Technically, it is already possible to sequence individual exomes or even genomes with the current NGS platforms, and it is being done for research in great numbers already,” he said.

In each case, and especially in a diagnostic setting, one has to make sure that interpretation is sound and to the benefit of the patient, insists Prof Bolz.

“Genetic counselling before sequencing of whole exomes or even genomes will be even more important than before running gene panels for a particular indication. For example, the patient will have to state whether she or he wants to be informed about incidental findings. Before exome/genome sequencing can become a diagnostic routine, frameworks that include appropriate consent forms among other things, for such large-scale NGS applications have to be set up,” he said.

With the advent of NGS in diagnostics, the overlap with research is larger than ever before, concludes Prof Bolz.

“I think it is crucial that the investigator has a good background knowledge about the genes and diseases analysed in order to get the right conclusion,” he said.

Structure and in whom vision is not limited by amblyopia. This is the likely scenario for future gene therapy treatments for most retinal diseases, as it will almost certainly be better to intervene to correct the underlying gene defect before the onset of retinal degeneration,” said Prof MacLaren.

Another interesting by-product of the choroideremia trial is that it has gone some way towards confirming what Prof MacLaren has advocated for some time—that treatment of the fovea is indeed possible in gene therapy.

“These are early days, but we have noted from our study that the human fovea can be detached before the onset of significant degeneration and exposed to therapeutic levels of AAV vector without significant detrimental effect. There will be complications as with all surgery to the retina, but the principle is there—it can be done. When analysing results from all studies, it is important not to confuse surgical complications with those arising due to properties of the vector,” he said.

Although this first trial will hopefully answer some of the key questions relating to the safety of gene therapy, many more questions concerning the benefits of early intervention, optimal dosing and surgical technique remain to be elucidated, added Prof MacLaren.

“Ideally we would like to treat patients as early as possible to preserve their remaining sight. Although the gene therapy appears to replace the missing REPI protein effectively, we still do not know how long the effect will last. Of course we hope that humans share the same features of retinal gene therapy in the animal models studied so far in which the effects are long lasting, but we cannot be sure until we follow these patients up for years. We also do not know the optimal dose of gene therapy and most importantly we need to develop an effective surgical technique that can be used widely in many centres without complications,” he said.

Other trials on the horizon

As the most common juvenile degenerative retinal disease, Stargardt’s disease is caused by a mutation of the ABCR gene that leads to the degeneration of photoreceptors in the retina and vision loss.

Up to 28 people are currently being enrolled in a Phase I/IIa clinical trial to be conducted at Oregon Health and Science University in Portland, Oregon, US and at the Centre Hospitalise Nationale D’Ophthalmologie des Quinze-Vingts in Paris, France.

The trial will assess the safety and efficacy of StarGen™ gene therapy (Oxford BioMedica), which uses Lentivector® technology, a “stripped-down” version of the equine infectious anaemia virus (EIAV), to deliver a corrected version of the ABCR gene.

The same company is also currently recruiting for trials of UshStart®, its gene therapy targeted for the treatment of RP associated with Usher’s syndrome type 1B.

The first human trials for patients with achromatopsia are also on the horizon.

While achromatopsia has often been loosely defined as colour blindness, the disease is actually a lot more debilitating than the term implies, points out Prof Martin Biel, a pharmacologist at Ludwig-Maximilians Universität in Munich, Germany.

“Achromatopsia is a lot more than just colour blindness. It is a strictly autosomal recessive or sporadic disease characterised by a loss of cone function and patients lack colour discrimination, have very poor visual acuity, pendular nystagmus and severe photophobia. Its prevalence is about one
cone degeneration had already started. Cone photoreceptors in mice even though that it is feasible to rescue fast degenerating cone photoreceptors in mice even though cone degeneration had already started.

Most cases of achromatopsia are due to a mutation in one or other of the CNGA3 and CNGB3 genes essential for photoreceptive function in cells. Prof Biel in collaboration with Prof Mathias Seeliger of Tübingen University and Dr Tim Gollisch of the Max Planck Institute for Neurobiology showed that it is feasible to rescue fast degenerating cone photoreceptors in mice even though cone degeneration had already started.

But will the same treatment work in humans? “What we need now are robust clinical data,” Prof Biel told EuroTimes. “We simply do not know so far to what extent vision can be restored in patients. We also do not know whether vision can be improved in the long term in patients. These questions can be only answered on the basis of the clinical studies which are about to start in several countries,” he said.

While not wanting to raise expectations to unsustainable levels, Prof Biel remains “very optimistic” that gene therapy of achromatopsia, and to a lesser extent RP, will also deliver positive results in humans. “I am particularly optimistic with regard to the treatment of achromatopsia because the functionally impaired cone photoreceptors survive for quite a long time in these patients. Hence there is a large therapeutic window for the application of viral vectors. RP is more complicated however, since depending on the affected gene neurodegeneration can proceed very fast. A lot will depend on the extent to which the neurodegenerative process can be stopped or slowed down,” he said.

As research continues to advance on all fronts – gene sequencing, diagnostics, patient selection, delivery methods, surgical techniques and so forth – and the trial data continues to accumulate, there is a real sense that gene therapy may be on the cusp of finally achieving its promise.

“I am very optimistic with many groups now working on improving retinal gene therapy. I feel that a real breakthrough could happen in a few years,” said Prof Biel. “These are early days, but we now have a second genetic retinal disease showing a promising response to gene therapy. I would urge all clinical retinal specialists to engage with this technology,” said Prof MacLaren.

“We are now into the domain of optimising the surgical approach and defining the clinical observations that are needed to select the most appropriate patients and assess treatment effects,” he concluded.

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