Let There Be Light: Gene and Cell Therapy for Blindness

Deniz Dalkara,^{1,*} Olivier Goureau,¹ Katia Marazova,¹ and José-Alain Sahel^{1–3}

¹Sorbonne Universités, UPMC Université Paris 06, INSERM, CNRS, Institut de la Vision; ²Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, DHU Sight Restore, INSERM-DHOS CIC 1423; and ³Fondation Ophtalmologique Adolphe de Rothschild, Paris, France.

Retinal degenerative diseases are a leading cause of irreversible blindness. Retinal cell death is the main cause of vision loss in genetic disorders such as retinitis pigmentosa, Stargardt disease, and Leber congenital amaurosis, as well as in complex age-related diseases such as age-related macular degeneration. For these blinding conditions, gene and cell therapy approaches offer therapeutic intervention at various disease stages. The present review outlines advances in therapies for retinal degenerative disease, focusing on the progress and challenges in the development and clinical translation of gene and cell therapies. A significant body of preclinical evidence and initial clinical results pave the way for further development of these cutting edge treatments for patients with retinal degenerative disorders.

INTRODUCTION

Blinding diseases of the retina

RETINAL DISEASES are a major cause of irreversible blindness. These conditions can be caused genetically or acquired later in life. Complex diseases have both genetic and acquired counterparts. Most common forms of multifactorial retinal diseases include macular degeneration, glaucoma, diabetic retinopathy, and retinoblastoma. Inherited retinal degenerations, on the other hand, are entirely linked to mutations in retinal neurons and their underlying epithelium. Retinal cell death is the main cause of vision loss in many blinding conditions, for which gene and cell therapy approaches offer intervention at various stages (see Fig. 1 for an example). Basic research on how and why retinal cells die in various diseases is crucial for the development of treatment strategies to prevent or reverse vision loss by gene and cell therapy. This review focuses on the latest developments in laboratory and clinical aspects of gene and cell therapy for retinal diseases.

Why the eye?

The eye represents an ideal target for gene and cell therapies: it is easily accessible and small (requiring a low volume of virus per active dose), highly compartmentalized (permitting various ocular tissues—anterior chamber, vitreous cavity, or

subretinal space—to be specifically targeted), and separated from the rest of the body by the blood–retinal barrier (ensuring ocular immune privilege and minimal systemic dissemination). As the retinal cells normally do not divide, the cell population remains stable, making it possible to use nonintegrating vectors for sustained transgene expression (for review, see References 1–3). Other important reasons why the eye has been at the forefront of gene and cell therapies is the fact that the contralateral eye can serve as an internal control, which is extremely helpful in evaluation of outcomes. Last, progress in imaging technologies (such as optical coherence tomography, adaptive optics) for visualizing this accessible part of the body has been of great value in both diagnostics and follow-up after treatments.

GENE THERAPY

Gene therapy is an emerging therapeutic approach to treat, cure, or prevent a disease by providing a gene with therapeutic action. Diseases associated with loss-of-function mutations can be treated by gene replacement therapy (also referred to as *gene supplementation*), whereas those associated with gain-of-function mutations require eradication of mutant alleles in addition to supplementing the gene. In all instances, the genetically modifying factors (DNA or RNA and/or their

*Correspondence: Dr. Deniz Dalkara, Institut de la Vision, 17 rue Moreau, 75012 Paris, France. E-mail: deniz.dalkara@gmail.com

Figure 1. Progression of a genetic disease through retinal cell death: autosomal dominant retinitis pigmentosa serves as an example. Healthy retina is composed of five neuronal types, with the photoreceptors being the primary neurons. In retinitis pigmentosa, mutations mostly in rod transcripts (shown in blue) lead to degeneration of these cells (stage I) followed by progressive cone (orange) degeneration (stages II and III). In the final stage (stage IV), all photoreceptor cells are lost, leaving the retina blind. Color images available online at www.liebertpub.com/hum

interacting proteins) need to be delivered into the relevant target cells. Advances in the design and production of gene delivery vectors have been an important part of gene therapy progress and are discussed below. In addition, advances in molecular genetics and rapidly evolving knowledge of retinal biology have allowed significant progress to be made in gene therapy for retinal disorders, with promising results not only in animal models, but also in humans.

Gene delivery: viruses, nanoparticles, physical methods

Most gene therapy studies use viral vectors, such as adenovirus (Ad), adeno-associated virus (AAV), or lentivirus (LV), to enable gene delivery to the retina. Two local administration routes allow viral vectors to access retinal cells. Viral vectors can either be injected into the vitreous cavity via an intravitreal injection, or they can be injected into the subretinal space, created through a transient retinal detachment. Intravitreal injections deliver the vector in proximity to the retinal ganglion cells and are the preferred delivery route for targeting the inner retina. Subretinal injections deliver vectors between the photoreceptors and their underlying retinal pigment epithelium (RPE). As most inherited retinal degenerations are caused by mutations found in the photoreceptor and RPE cells, subretinal injections have been used in most gene therapy studies.

Depending on the cell target, Ad, LV, and AAV have been studied. After subretinal delivery both

Ad and LV transduce the RPE efficiently. Ad, however, has been associated with cytotoxic T lymphocyte-mediated removal of the transduced cells that express the encoded Ad proteins,⁴ leading to transient gene expression. More recently, helper-dependent Ad vectors devoid of sequences encoding viral proteins have been developed and shown to target the RPE stably.^{5,6} Thus far, photoreceptor transduction remains elusive with Ad and LV vectors despite the great diversity of new serotypes and pseudotypes tested.⁷ Further development of Ads and discovery of new Ad serotypes might enable photoreceptor transduction with Ad^{8–10} in the future. If Ad can be modified to enable photoreceptor transduction, its large carrying capacity will be of interest for treating inherited retinal degenerations such as Usher syndrome (USH), in which the genes involved have very long open reading frames. LVs are an alternative to Ad. LV-based vectors are deleted of all viral genes and, thus, do not activate the immune system.^{11,12} However, LVs are integrating vectors, and this implies the possibility of insertional mutagenesis, potential mobilization in human cells, and vector replication.¹² Most studies showed that subretinal delivery of LVs leads to efficient RPE transduction,^{13–15} but postmitotic photoreceptors seem refractory to transduction by LV.^{16,17} The development of the nonprimate equine infectious anemia virus has raised hopes for overcoming this limitation.¹⁸ This has been the basis for the ongoing clinical trials to treat exudative age-related macular

degeneration (AMD) (ClinicalTrials.gov identifier, NCT01301443), Stargardt disease (STGD) (ClinicalTrials.gov identifier, NCT01367444), and USH type IB (USH1B) (ClinicalTrials.gov identifier, NCT01505062), using this vector. These clinical trials are discussed below. However, the transduction of mature neural retina remains a fundamental limitation of LVs and Ads as vectors for retinal gene therapy. This is one of the reasons why AAV has been the preferred vector of choice for gene delivery to the retina. AAVs have an excellent safety profile (lack of pathogenicity and low immunogenicity) and provide long-lasting transgene expression (for a review, see Colella and Auricchio¹⁹). AAV has the additional advantage of being a small virus, which can diffuse easily across biological barriers and within neural tissue. It is the only vector that can provide gene delivery to the inner retina after intravitreal delivery.^{20–22} Although the small size of the AAV particle is an advantage, it is also its weakness: the 25-nm AAV particles can package only 4.7 kb of genetic material, limiting its application in some diseases. Nevertheless, AAV has yet another advantage that has contributed to its development as a gene delivery vehicle: it is a nonenveloped virus and its capsid can be easily modified using genetic engineering techniques. As such, it has been extensively explored for its ability to target various groups of cells in the retina.^{23–28} As an example, it has been engineered to provide gene delivery into deeper layers of the retina after intravitreal administration, removing the need for subretinal detachment.^{23–25,29} One such AAV variant, called 7m8, was able to ensure efficient pan-retinal delivery of the therapeutic gene from the vitreous, with long-term histological and functional rescue of X-linked retinoschisis and Leber congenital amaurosis (LCA) phenotypes in mice and provided superior retinal gene delivery in nonhuman primates.²⁵

Past studies have pointed toward the higher efficiency of viral versus nonviral vehicles³⁰ for retinal gene delivery. However, reports on nanoparticle-mediated retinal gene therapy showed an improvement compared with previous studies with nonviral agents.³¹ Nonviral (lipid or nanoparticle) carriers provide a complementary approach (for a review, see Adijanto and Naash³²). These include naked DNA, DNA-encapsulating liposomes, and compacted-DNA nanoparticles (cationic liposome/DNA complexes). In general, they allow transfection of cells with larger pieces of DNA and carry lower risk of immune responses associated with viral gene delivery.^{33,34} However, lack of long-term gene expression is a major limitation of such vec-

tors. For example, clinical trials using polyethylene glycol-substituted 30-mer lysine peptide-based nanoparticles and lipid-mediated vectors to deliver therapeutic genes to the nasal mucosa of patients with cystic fibrosis reported no detectable gene expression, although vector DNA was detectable for at least 2 weeks.³⁵ Although their use has been limited thus far, their additional development for increased efficacy could make them versatile tools for gene delivery in the years to come.

The first clinical success of retinal gene therapy

Encouraging results from animal studies (mouse, rat, dog) showed that AAV-mediated gene therapy has the potential to slow down or reverse vision loss, and paved the way toward first application in humans. The first gene therapy success has been documented with the clinical trials for LCA, a severe retinal dystrophy characterized by visual impairment from birth.³⁶ It is caused by mutations in at least 19 different genes (22 mapped and identified genes; see the Retinal Information Network [<http://www.retnet.org>], accessed January 20, 2016). Mutations in the gene encoding the RPE-specific protein RPE65 appear to account for about 5–10% of LCA cases.^{37,38} For this specific form of human LCA (LCA2), the first clinical trial of gene replacement therapy started in 2007 at (1) the UCL Institute of Ophthalmology and Moorfields Eye Hospital (London, UK), (2) the Scheie Center for Hereditary Retinal Degenerations, University of Pennsylvania (Philadelphia, PA) and the University of Florida College of Medicine (Gainesville, FL), and (3) the Children's Hospital of Philadelphia and University of Pennsylvania, and the Second University of Naples and the Telethon Institute of Genetics and Medicine (TIGEM) (Naples, Italy). Patients received a single subretinal injection of AAV2 vector carrying the *RPE65* gene in the most affected eye. In 2008, the independently working groups reported the first safety and efficacy results of the AAV-mediated *RPE65* transfer.^{39–41} In addition to excellent safety, improvements in some measures of vision (including best-corrected visual acuity, kinetic visual field, nystagmus testing, pupillary light reflex, microperimetry, dark-adapted perimetry, dark-adapted full-field sensitivity testing) have been demonstrated in these phase I clinical trials. Since then, results of follow-up and dose-escalation studies have been published^{40,42–46} and confirmed the feasibility and benefits of gene therapy in retinal degenerative diseases. In view of these encouraging results, readministration of *RPE65* gene-based treatment was performed for

the first time in the contralateral eye of adult patients with LCA, 3 years after the initial gene therapy administration.⁴⁷ This intervention led to positive improvements in the second eye. In addition to improved visual outcomes, functional magnetic resonance imaging (fMRI) studies provided evidence that the human visual cortex responds to gene therapy-mediated recovery of retinal function.⁴⁵ fMRI evaluation found correlation between preserved light sensitivity and a cortical projection zone of pseudo-foveas developed in treated retinal regions (observed 9–12 months after therapy and persisting for up to 6 years).⁴⁸ Multimodal noninvasive neuroimaging has revealed long-term structural plasticity in the visual pathways of patients with LCA who received single-eye gene augmentation therapy.⁴⁹ It has been suggested that the visual experience gained by gene therapy may promote reorganization and maturation of synaptic connectivity in the visual pathways of the treated eye in patients with LCA. Today, retinal gene therapy has entered into phase III (ClinicalTrials.gov identifier, NCT00999609). At least 24 patients with LCA (age, 3 years or older), will be recruited at either the Children's Hospital of Philadelphia or the University of Iowa (Iowa City, IA). They will receive a subretinal administration of AAV2-*RPE65* to both eyes. A prospective open label gene therapy (AAV4-*RPE65*) study for *RPE65*-associated retinal dystrophy was also performed and completed in the Nantes University Hospital (Nantes, France) (ClinicalTrials.gov identifier, NCT01496040) (results still to be published).

The initial positive results from LCA gene therapy studies were challenged by new findings. In 2013, two groups^{50,51} reported that early visual improvements in *RPE65*-treated patients with LCA persist up to 3 years, with no detectable decline in visual improvement. However, retinal degeneration continued to progress. This observation was also seen in *RPE65*-mutant dogs. Two years later, the *New England Journal of Medicine* published the long-term follow-up results of two independent studies on *RPE65* gene therapy. Jacobson and colleagues (United States) described follow-up data from three *RPE65*-treated patients.⁵² The patients all had improvement in visual sensitivity in the treated region that was sustained between 1 and 3 years after gene therapy. Unexpectedly, 4.5–6 years after treatment, the areas of improved vision were found to have progressively diminished in all three patients. The authors concluded that the degeneration continued at the same rate as in untreated retina, despite the initial improvement. The study from the United Kingdom (Bainbridge

and colleagues)⁵³ involved 12 patients (in this study the fovea was also targeted in order to improve both central and extrafoveal vision). Six of these patients had improvements in visual sensitivity that peaked 6–12 months after treatment. Similarly to the U.S. study, the effect declined or was lost by 3 years postinjection. These new findings prove that at least for several years gene therapy can improve vision but also indicate that the photoreceptors continue to die after the peak improvement, regardless of treatment.

As of today, it is not clear what caused the effects of gene therapy to be transient in these two clinical studies. The study by Bainbridge and colleagues⁵³ concluded that there is a species difference in the amount of *RPE65* required to drive the visual cycle and that the demand for *RPE65* in affected persons in their study was not met to the extent required for a durable, robust effect (“too little” therapeutic protein). Indeed, the demand for *RPE65* is likely higher in humans than in dogs.⁵⁴ Another potential culprit for the transient effects is related to the stage of the retina at the time of intervention and progressive loss of trophic support (especially regarding the cones). The study by Jacobson and colleagues⁵² speculated that healthier photoreceptors survived in the treated retina, whereas other, more stressed rods were already in a preapoptotic state (“at the point of no return”) and continued to die. The loss of visual function at later times after treatment is in line with this natural progression of degeneration. Furthermore, the reduction in the number of rod photoreceptors in spite of therapy may eventually have led to a loss of trophic support for the cone photoreceptors that initially responded to therapy. In both studies, there were no improvements in foveal function despite vector having been delivered to the fovea in some of the patients. The question concerning why *RPE65* gene supplementation improves the function of extrafoveal, but not foveal, cones remains unresolved but might well be a long-term complication of surgery. Indeed, connections between the RPE and the cones are different in the fovea and at the periphery. Although successful results have been obtained in the macula in gene supplementation therapy for choroideremia,^{55,56} it has been proposed that detaching the foveal cones has detrimental consequences in LCA.⁴⁶

Whatever might be responsible for the reported transient effects, there is a clear need to improve the initial approaches. One study⁵⁷ suggests, at least in retinitis pigmentosa (RP) due to mutant rod-specific cyclic GMP (cGMP) phosphodiesterase 6b (PDE6b), that photoreceptor cell death can be

halted, no matter at what disease stage the gene therapeutic intervention is provided. It is unclear whether the findings of this study also apply to LCA, but it is noteworthy that with an appropriate amount of therapeutic protein delivered to all mutant cells, one can stop the course of cell loss, challenging the “point of no return” hypothesis for photoreceptor cell death. One need for refinement is to better understand the visual cycle of human foveal cones and their reaction to detachment in order to make the treatments efficacious for visual acuity. Another need, identified through structural studies in dogs and patients, would be to seek a more complete therapeutic outcome that involves both visual improvement and structural rescue. A combination gene therapy in which trophic support is provided at the same time as gene supplementation might prove most effective in the long run. Further developments in vector systems that can deliver genes to the foveal region without the need for subretinal detachment will likely be beneficial for therapeutic outcome.^{24,25}

Implementation of gene therapy for other inherited retinal diseases

Today, gene therapy is being implemented for other retinal degenerative diseases. Positive outcomes have been published for the treatment of choroideremia, using AAV as a vector.^{55,56} These and other ongoing studies are discussed below. Further along in the pipeline are gene therapies for other forms of LCA caused by mutations in various genes. *GUCY2D* is one of the most frequently mutated genes (12%) and responsible for the LCA1 disease form.³⁶ Studies provided evidence that AAV-mediated subretinal delivery of *Gucy2e* preserves photoreceptor morphology and restores retinal function in mouse models over a lifetime,^{58,59} suggesting that gene replacement therapy for people with the LCA1 gene could be feasible. Orphan designation (EMA/COMP/97253/2014) for the development of AAV vector serotype 8 carrying the human *GUCY2D* gene was granted for the treatment of LCA1.⁶⁰ As mutations in *GUCY2D* are also associated with autosomal recessive forms of cone-rod dystrophy (reviewed in Boye et al.⁵⁹), this gene replacement therapy may offer vision restoration to a larger group of patients. Although preliminary, strategies for the development of gene therapy for *CEP290*-associated LCA (LCA10) are also under consideration. Burnight and colleagues⁶¹ provided evidence that LV vector expressing full-length human *CEP290* can correct the *CEP290* disease-specific cellular phenotype in patient-derived fibro-

blasts, but it is not clear whether the LV-mediated approach will be able to deliver to photoreceptors in patients. Another approach might be the use of CRISPR/Cas9-mediated gene editing; this is currently being developed by Editas (Cambridge, MA). Potential treatment of LCA4 due to *AIPL1* mutations is also under consideration.⁶² A high level of *AIPL1* photoreceptor expression and no toxicity were documented in *Aipl1* null mice and porcine eyes that received subretinal administration of AAV2/8-*AIPL1*.⁵⁴ As some patients with *AIPL1*-associated disease have a late onset and slow progression rate, it may be a good candidate for gene augmentation therapy.⁶³

Current clinical trials

Stargardt disease (STGD) is the most common hereditary macular dystrophy and the most common cause of central visual loss in young people. In the majority of cases (90–95%), the disease is inherited as an autosomal recessive trait and is associated with mutations in the photoreceptor-specific *ABCA4* gene (which encodes the ATP-binding cassette transporter involved in the clearance of retinoid by-products).⁶⁴ Proof-of-concept studies in *Abca4*^{-/-} mice⁶⁵ demonstrated that subretinal administration of LV vector carrying the human *ABCA4* gene was associated with reduced A2E accumulation, corrected lipofuscin levels, and improved RPE morphology and retinal function. On the basis of these findings, the first gene-based therapy clinical trial for the treatment of STGD moved into human studies. Work with SAR422459 (LV-*ABCA4*) is currently underway (ClinicalTrials.gov identifier, NCT01367444) at the Casey Eye Institute, Oregon Health and Science University (Portland, OR) and the National Eye Hospital of Quinze-Vingts (Paris, France). No serious adverse events related to dose level 1 or the method of administration have been reported so far (Data Safety Monitoring Board, 2012; see <http://www.oxfordbiomedica.co.uk/press-releases/oxford-biomedica-announces-positive-dsmb-review-of-ongoing-retinostat-r-and-stargen-clinical-studies/>). More recently, dual AAV systems have also successfully been implemented in bringing a gene therapy solution to Stargardt disease.⁶⁶ Subretinal delivery of *ABCA4* via optimized DNA–nanoparticles also resulted in persistent transgene expression and significant structural and functional correction in *Abca4*^{-/-} mice,³¹ suggesting a relevant alternative approach for *ABCA4* gene delivery.

Choroideremia (CHM) is an X-linked recessive disease that leads to progressive retinal degeneration and blindness caused by mutations in

the *CHM* gene. The first clinical trial for this monogenic retinal disorder without extraocular manifestations has been undertaken at Oxford University (Oxford, UK) to assess the safety and tolerability of the AAV2.*REP1* vector administered at two different doses to the retina in 12 patients with CHM (ClinicalTrials.gov identifier, NCT01461213). So far, no major safety issues have been reported⁵⁵ and some improvements above baseline were reported.

X-linked retinoschisis (XLRS) is characterized by a splitting of the neurosensory retina and progressive macular atrophy. Proof of principle for gene replacement therapy (AAV8-*RS1*) in mouse models has been achieved for both structural and functional recovery,⁶⁷ and the first gene therapy trial is now being undertaken by the Sieving laboratory (National Eye Institute, National Institutes of Health, Bethesda, MD) (ClinicalTrials.gov identifier, NCT02416622) and by AGTC (Alachua, FL) (ClinicalTrials.gov identifier, NCT02416622).

Leber hereditary optic neuropathy (LHON) is a maternally inherited disease caused by mitochondrial DNA point mutations in complex I and characterized by acute (or subacute) painless loss of central vision resulting from degeneration of the retinal ganglion cell layer and optic nerve. Replacement of normal *ND4* and *ND1* gene transcripts in the fibroblasts of patients harboring mutations in these genes restored electron transport chain activity, and intravitreal viral delivery of normal gene rescued vision in an animal model of LHON.^{68–71} First-in-human dose escalation safety studies have been completed or are ongoing in several centers (ClinicalTrials.gov identifiers, NCT01267422, NCT02161380, and NCT02064569). No serious adverse reactions related to the treatment or the study procedures have been documented (Feuer et al.⁷² and Uretsky et al.⁷³). Preparation for the upcoming pivotal phase III of drug development will be undertaken.

There are other examples of ongoing and completed gene therapies for retinal diseases, including Usher syndrome 1b (MYO7A) (UshStat; ClinicalTrials.gov identifier, NCT01505062; the Institut de la Vision/Clinical Investigation Center at the National Eye Hospital of Quinze-Vingts and the Casey Eye Institute) and autosomal recessive retinitis pigmentosa caused by *MERTK* mutations (AAV2-VMD2-*hMERTK*). In 2013, an AAV vector carrying the human *CNGB3* gene received orphan drug designation (EU/3/13/1099) for the treatment of achromatopsia. AGTC plans to treat both *CNGB3* and *CNGA3* forms of achromatopsia.

Beyond gene supplementation

Secretion of antiangiogenic factors. Age-related macular degeneration (AMD) is the most frequent cause of vision impairment among the elderly. Wet AMD accounts for 90% of AMD-related blindness in these patients. The majority of current treatments for wet AMD aim to prevent choroidal neovascularization through the delivery of antiangiogenic factors (i.e., bevacizumab [Avastin] or ranibizumab [Lucentis]). These compounds inhibit vascular endothelial growth factor A (VEGF-A), which is thought to be responsible for the growth and increased permeability of new blood vessels.⁷⁴ As AMD is a complex disease, it was not considered a likely candidate for gene therapy. However, the success of VEGF antagonists, which required frequent readministration, and the possibility of long-term expression of antiangiogenic molecules through AAV-mediated expression sparked interest. In two ongoing phase I clinical trials, pigment epithelium-derived factor (PEDF) and soluble Fms-like tyrosine kinase 1 (sFLT) are being tested as potential candidates for the treatment of wet AMD (ClinicalTrials.gov identifiers, NCT01024998 and NCT01494805). All of the previously mentioned clinical trials are summarized in Table 1.

Neuroprotection. Neuroprotective agents can prevent and reverse oxidative stress and its damaging effects, and restore normal cell function. One retina-specific trophic factor, called rod-derived cone viability factor (RdCVF), has been shown to induce cone survival and functional rescue in animal models of retinitis pigmentosa (RP),^{75,76} in a gene-independent manner. AAV-RdCVF prolonged cone survival and function in RP mice.⁷⁷ It has been suggested as a particularly well-suited therapy for preventing secondary cone degeneration in rod–cone dystrophies and for treating RP at a stage of night blindness associated with moderate central visual impairment (stages I and II in Fig. 1).⁷⁸ One study showed that retinal cone survival promoted by RdCVF is associated with accelerated glucose entry into photoreceptors and enhanced aerobic glycolysis, uncovering an entirely novel mechanism of neuroprotection.⁷⁹ Structural and functional rescue in retinal diseases has also been reported for intraocular gene transfer of other vector-delivered neuroprotective factors in preclinical animal models, for example, ciliary neurotrophic factor (CNTF),^{80–82} PEDF,⁸³ and glial-derived neurotrophic factor.⁸⁴ Although promising, no clinical trials have thus far been conducted in which trophic factors are provided in the form of gene therapy. Encapsulated cell technology has been used in

Table 1. Current clinical trials in retinal gene therapy

Disease	Encoded gene	Protein	Cell target	Vector	Clinical trial number
LCA2	<i>RPE65</i>	Retinal isomerase RPE65	RPE	AAV	NCT00516477 NCT00749957 NCT00821340 NCT01208389 NCT00999609 NCT00481546 NCT00643747
Choroideremia	<i>CHM1</i>	REP1	RPE + choroid	AAV	NCT02077361 NCT01461213 NCT02341807 NCT02553135 NCT02407678
Usher syndrome	<i>MYO7A</i>	Myosin 7a	PR	Lentivirus	NCT01505062 NCT02065011
Stargardt disease	<i>ABCA4</i>	Photoreceptor-specific adenosine triphosphate-binding cassette transporter	PR	Lentivirus	NCT01367444 NCT01736592
LHON	<i>ND4</i>	NADH dehydrogenase	RGC	AAV	NCT01267422 NCT02064569 NCT02161380
arRP	<i>MERTK</i>	MER tyrosine kinase receptor	RPE	AAV	NCT01482195
Retinoschisis	<i>RS1</i>	Retinoschisin	PR	AAV	NCT02317887 NCT02416622
AMD	<i>FLT1</i>	Soluble FMS-like tyrosine kinase receptor	Soluble	AAV	NCT01494805 NCT01024998
	<i>PLG</i> and <i>COL18A1</i>	Endostatin and angiostatin Angiostatin and endostatin	Soluble	Lentivirus Lentivirus	NCT01301443 NCT01301443 NCT01678872

AAV, adeno-associated virus; AMD, age-related macular degeneration; arRP, autosomal recessive retinitis pigmentosa; LCA2, Leber congenital amaurosis type 2; LHON, Leber hereditary optic neuropathy; PR, photoreceptor; REP1, Rab escort protein 1; RGC, retinal ganglion cell; RPE, retinal pigment epithelium.

ongoing and completed clinical trials to provide neuroprotection through CNTF secretion in atrophic macular degeneration (NCT00447954), retinitis pigmentosa (NCT00447980, NCT00447993), achromatopsia (NCT01648452), macular telangiectasia (NCT01949324), and glaucoma (NCT01408472).

Once photoreceptors stop capturing light: What next?

When photoreceptor degeneration is too advanced (stages III and IV in Fig. 1), patients will have little chance to benefit from gene replacement therapy or neuroprotection. In these cases, new strategies for vision restoration should be explored. These include retinal prostheses—designed to stimulate responses from surviving inner retinal neurons^{85–87}—or optogenetics, a technique allowing control of neural activity via genetic introduction of light-sensitive proteins such as channelrhodopsin and halorhodopsin.^{88–91} Vertebrate opsins such as melanopsin⁹² and rhodopsin,^{93,94} as well as a chimera between melanopsin and mGluR6 receptor,⁹⁵ have also been used for vision restoration in late-stage RP. The common feature between these approaches is the use of a gene encoding a light-

sensitive protein that transforms light-insensitive cells of the retina into artificial photoreceptors. This strategy has enjoyed success in preclinical studies, in a number of rodent models of inherited retinal disease. At present, optogenetics is being moved toward the clinic by several companies (GenSight Biologics [Paris, France] and RetroSense Therapeutics [Ann Arbor, MI]) that have shown interest in the use of microbial opsins for vision restoration. Evaluation of candidate patients for optogenetic therapy is also underway.⁹⁶

CELL THERAPY

Cell therapy represents an alternative to repair the degenerated retina. Transplantation of retinal cells has been historically viewed as a potential vision restoration strategy for retinal degenerative diseases, particularly in disease stages associated with significant cell damage (stages II and IV in Fig. 1). This therapeutic approach aims at replacing the lost retinal cells using stem cells, progenitor cells, and mature neural retinal cells. The main advantage of cell therapies as a source for regenerative therapy is that they are mutation-independent and can be used in a wide range of

retinal degenerative conditions. Patients with retinal degeneration typically lose RPE cells, photoreceptors, or both. Therefore, two main cell sources can be considered: first, RPE cells to replace dysfunctional or degenerated RPE and to prevent photoreceptor cell loss and, second, photoreceptor precursors to repair the degenerating neural retina.

Several novel stem cell-based therapies addressing inherited and age-related retinal degenerative diseases are currently under development and/or clinical evaluation. They are based on a significant body of evidence showing that human pluripotent stem cells (PSCs) can be expanded indefinitely in culture and can be used as an unlimited source of retinal cells (RPE cells, photoreceptors, and retinal ganglion cells) for the treatment of retinal degeneration. Since their first establishment in 1981,⁹⁷ human embryonic stem cells (ESCs) have been intensively studied by many groups worldwide. Furthermore, the discovery that somatic cells can be reprogrammed into an ESC-like pluripotent state, known as induced pluripotent stem cells (iPSCs), offers the same applications in regenerative medicine, bypassing human ESCs, the use of which has major ethical restrictions. After reprogramming mouse somatic cells into iPSCs,⁹⁸ the Yamanaka group was able to reprogram human fibroblasts into iPSCs by overexpressing the four transcription factors OCT4, KLF4, SOX2, and C-MYC.⁹⁹ Today, these two types of PSCs represent major cell sources in regenerative medicine.

Various groups have reported encouraging morphological and functional results in animal models of retinal degeneration after transplantation of RPE cells, retinal progenitor cells, photoreceptor precursors, or full-thickness retinal sheets (for reviews, see References 100–103). Integration into the host retina and reconstruction of functional neural circuitry have been seen as major hurdles for successful cell transplantation. For these reasons, the most advanced studies today concern the transplantation of RPE.

Cell transplantation using human PSCs

Human PSCs for RPE cell replacement. At present, the most plausible approach for the development of cell therapy for macular degeneration consists of replacement of lost or dysfunctional RPE with healthy RPE cells, which are essential for photoreceptor shedding, maintenance, and survival. Indeed, various groups have already demonstrated that human PSCs can be differentiated into RPE cells with morphological and functional characteristics similar to those of human RPE cells (for review, see Leach and Clegg¹⁰⁴).

The first-in-human safety and tolerability prospective clinical trial to evaluate subretinal injection of human ESC-derived RPE cells (specifically line MA09-hRPE) in patients with dry AMD and STGD is currently underway. It has been sponsored by Ocata Therapeutics (Marlborough, MA; formerly Advanced Cell Technology) and conducted at four centers in the United States: Jules Stein Eye Institute (University of California Los Angeles, Los Angeles, CA), Wills Eye Hospital (Philadelphia, PA), Bascom Palmer Eye Institute (Miami, FL), and Massachusetts Eye and Ear Infirmary (Boston, MA). Doses of 50,000, 100,000, and 150,000 cells (cell suspensions) have been administered to one eye of each of 9 patients with dry AMD and 9 patients with STGD (3 patients in each cohort). Patches of increasing subretinal pigmentation consistent with the transplanted RPE cells were documented in 13 of the 18 patients (72%), but were not correlated with visual acuity improvement. Follow-up testing showed that 10 of 18 treated eyes had substantial improvements in the first year after transplantation. Stable improvement of visual acuity over 22 months was reported in 7 patients, but decreased by more than 10 letters in one patient. Untreated eyes did not show similar visual improvements, but no correlations between visual acuity improvement and the number of transplanted cells was reported.^{105,106} Median follow-up at 22 months suggested no major safety concerns (no signs of hyperproliferation, tumorigenicity, ectopic tissue formation, or apparent rejection). Adverse events were associated with surgery and immunosuppressive treatment but were not considered related to the human ESC-derived cells.^{105,106} These results provide evidence of the medium- to long-term safety, graft survival, and biological activity on injection of human ESC-derived RPE cell suspensions into patients with macular degeneration.

To date, at least 15 ongoing clinical trials are registered at the International Clinical Trials Registry Platform (ICTRP) of the World Health Organization to test stem cell-based replacement therapies for treatment of retinal dystrophies (Table 1). Some examples include the phase I/II clinical trials with human ESC-derived RPE cells sponsored by CHA Biotech (Seoul, South Korea), Cell Cure Neurosciences (Jerusalem, Israel), and Pfizer (Tadworth, U.K.).¹⁰⁷ The London Project to Cure Blindness (sponsored by Pfizer) will insert a monolayer sheet of human ESC-derived RPE cells cultured on polyester membrane into 10 patients with wet AMD and rapid recent vision decline. This polyester matrix has been reported to maintain

polarized human RPE cells after grafting into the rabbit subretinal space.¹⁰⁸ Similarly, the California Project to Cure Blindness will use a differentiated polarized monolayer of RPE cells attached to a nondegradable Parylene membrane possessing the permeability properties of a healthy Bruch's membrane. A human phase I/II clinical trial will evaluate the safety and tolerability of this tissue-engineering product (TEP) in patients with gyrate atrophy.^{101,109} The proof of concept with this TEP has been reported in RCS rats and in Yucatan pigs.^{101,110}

As human iPSCs can be obtained directly from the patient, they have the advantage of being autologous and therefore less immunogenic than ESCs for future cell transplantation studies. In this context, the Takahashi group at the RIKEN Center for Developmental Biology (Kobe, Japan) is currently setting up human clinical trials with human iPSC-derived RPE for the treatment of AMD. On the basis of safety studies in rodents and monkeys, this group started to implant a sheet of RPE differentiated from iPSCs previously derived from the fibroblasts of one patient with an exudative form of AMD.¹¹¹ This pilot clinical study is assessing the safety (i.e., the potential to induce immune reactions, cancerous growth) and feasibility of the transplantation of autologous iPSCs. No further details have yet been reported.

Human PSCs for photoreceptor cell replacement. Whereas RPE replacement alone may be used for specific disease indications, transplantation of photoreceptors—as retinal sheet or as suspension of dissociated cells—is required after extensive photoreceptor degeneration. Retinal neurons, including photoreceptors, have been generated from human iPSCs by various laboratories worldwide (for reviews, see Borooah et al.¹¹² and Chen et al.¹¹³), but so far cell transplantation to restore neural retina is restricted to animal models. Prior groundbreaking studies in mice revealed that the ontogenetic stage of transplanted cells is crucial for successful integration into the adult host retina and recovery of vision.^{114–116} Indeed, the Ali group demonstrated in mice that only stage-specific photoreceptor precursors, corresponding to postmitotic committed photoreceptors, are able to efficiently integrate into the degenerating retina, differentiate into mature photoreceptors, form synaptic connections, and possibly lead to recovery of visual function.^{114,116} Furthermore, a direct relationship between cellular integration and functional recovery has been clearly established.¹¹⁶ From these pioneering studies

emerged the importance of identifying the *in vitro* equivalent of postmitotic postnatal photoreceptor precursors derived from PSCs. These photoreceptor precursor cells (PPCs), derived from mouse ESCs in which green fluorescent protein (GFP) allowed tracing of the photoreceptor lineage, have been successfully differentiated and transplanted into the mouse retina.^{117,118} To date only one study has reported transplantation of photoreceptors derived from human PSCs in mouse models of photoreceptor degeneration.¹¹⁹ After subretinal transplantation of virally labeled GFP-positive photoreceptors, Lamba and colleagues¹¹⁹ demonstrated cell integration into the remaining neural retina and partial restoration of visual function. The use of fluorescent reporter cell lines to isolate photoreceptor precursors is not compatible with future clinical applications. The surface antigen CD73, previously used to isolate precursors of photoreceptors from mouse postnatal retina for transplantation,^{120,121} could be a promising candidate. Our group demonstrated that photoreceptor precursors differentiated from human iPSCs specifically expressed CD73.¹²² On the basis of data with mouse ESCs, the use of a five-cell surface biomarker panel (CD73, CD133, CD47, and CD24 [positive] and CD15 [negative]) could be used to improve the isolation of photoreceptor precursors.¹²³

In the case of severe degenerations and loss of the outer nuclear layer (ONL), transplantation of retinal sheets rather than dissociated cells could be required. The Takahashi group transplanted mouse PSC-derived retinal sheets containing a defined ONL into *rd1* mice (a model of advanced retinal degeneration associated with lost ONL) and observed host-graft synaptic connections.¹²⁴ The development of innovative protocols allowing the generation of neuroretinal structures from human PSCs^{122,125,126} will be helpful in assessing the capacity of human retinal sheet to make contact with the recipient retina after subretinal transplantation.

Alternative source of human cells for retinal cell replacement. It has been reported that many types of stem cells, such as neural stem cells (NSCs) and mesenchymal stem cells (MSCs), possess inherent neuroprotective properties when transplanted into animal models of retinal disease (for review, see Bull and Martin¹²⁷). Even though the generation of new retinal cells directly derived from MSCs and NSCs remains unlikely, paracrine effects (such as antiapoptotic and antiinflammatory signaling) could explain how these cells contribute to prolonged retinal cell survival. StemCells (Newark,

CA) is sponsoring a study to determine the safety and potential benefits of subretinal injection of human NSCs into patients with geographic atrophy due to AMD. The trial is based on findings that subretinal transplantation of human NSCs (grown as neurospheres) derived from the fetal brain can partially protect against photoreceptor degeneration and preserve the visual function in RCS rats.¹²⁸ Early results at 6 months' follow-up showed maintenance or improvement in best corrected visual acuity and contrast sensitivity with no safety concerns.¹²⁹ Autologous bone marrow-derived stem cells (BMSCs) are under evaluation in patients with AMD in phase I/II clinical trials in southern Florida and California and in various locations worldwide (Table 2). Safety studies involving the subretinal administration of human umbilical tissue-derived cells (UTCs; CNTO 2476) are currently being performed in patients with advanced RP and in subjects with visual acuity impairment associated with geographic atrophy secondary to AMD. No clinical data have yet been reported. A similar concept is being implemented in the RP project of the California Institute for Regenerative Medicine (Oak-

land, CA), in conjunction with jCyte (Newport Beach, CA). They will explore the safety of intravitreal injection, into patients with RP, of human retinal progenitor cells obtained from fetal retina and expanded in culture. It is expected that these cells will not only exert neurotrophic support but also will differentiate and integrate into the retina.

Another type of cell-based therapy for retinal degeneration approaching clinical translation is the use of human cells to provide neurotrophic factors in order to improve the survival of photoreceptors and their function. An example in this respect is the implantable cell-encapsulation device NT-501 developed by Neurotech Pharmaceuticals (Cumberland, RI)¹³⁰ that consists of a human RPE cell line transfected with a plasmid encoding CNTF encapsulated within a semipermeable polymer membrane and supportive matrices. A phase I clinical trial indicated that CNTF is safe for the human retina even with severely compromised photoreceptors and may have application beyond disease caused by genetic mutations.¹³¹ At present, the device is under evaluation in phase II studies for the treatment of dry AMD and RP.

Table 2. Clinical trials using stem cell-based therapies in the retina: initiated or completed as of November 2015

Sponsor (location)	Identifiers	Cell source	Target disease	Delivery method	Phase
Ocata Therapeutics (USA, UK)	NCT01345006/NCT02445612	hESC-derived RPE cells	Stargardt disease	Subretinal	I/II
	NCT01344993/NCT02463344/ NCT02563782	hESC-derived RPE cells	Dry AMD	Subretinal	I/II and II
	NCT02122159	hESC-derived RPE cells	Myopic macular degeneration	Subretinal	I/II
CHA Biotech (South Korea)	NCT01674829	hESC-derived RPE cells	Dry AMD	Subretinal	I/II
Cell Cure Neuroscience (Israel)	NCT02286089	hESC-derived RPE cells	Dry AMD	Subretinal	I/IIa
Pfizer/University College London (UK)	NCT01691261	hESC-derived RPE cell sheets	Wet AMD	Subretinal	I
RIKEN Institute (Japan)	UMIN000011929	hiPSC-derived RPE cell sheets	Wet AMD	Subretinal	I
jCyte (USA)	NCT02320812	hRPCs	Retinitis pigmentosa	Subretinal	I
StemCells (USA)	NCT01632527	hNSCs	AMD	Subretinal	I
	NCT02467634	hNSCs	GA and AMD	Subretinal	II
	NCT01920867	hBMSCs	AMD, hereditary retinal dystrophies and glaucoma	Intravitreal and subretinal	N/A
Retina Associates of South Florida (USA)					
University of São Paulo (Brazil)	NCT01518127	hBMSCs	AMD and Stargardt disease	Intravitreal	I
	NCT01068561/ NCT01560715	hBMSCs	Retinitis pigmentosa	Intravitreal	I
University of California Davis (USA)	NCT01736059	hBMSCs	AMD, diabetic retinopathy, retina vein occlusion, retinitis pigmentosa, hereditary macular degeneration	Intravitreal	I
Red de Terapia Celular (Spain)	NCT02280135	hBMSCs	Retinitis pigmentosa	Intravitreal	I
Chaitanya Hospital (India)	NCT01914913	hBMSCs	Retinitis pigmentosa	Intravitreal	I/II
Al-Azhar University (Egypt)	NCT02016508	hBMSCs	AMD	Intravitreal	I/II
Mahidol University (Thailand)	NCT01531348	hBM-derived MSCs	Retinitis pigmentosa	Intravitreal	I
Janssen Research & Development (USA)	NCT00458575	hUTCs	Retinitis pigmentosa	Subretinal	I
	NCT01226628	hUTCs	AMD	Subretinal	I/II

AMD, age-related macular degeneration; GA, geographic atrophy; hBMSCs, human bone marrow-derived stem cells; hESC, human embryonic stem cell; hiPSCs, human induced pluripotent stem cells; hNSCs, human neural stem cells; hRPCs, human retinal progenitor cells; hUTCs, human umbilical tissue-derived stem cells; MSCs, mesenchymal stem cells; N/A, not available; RPE, retinal pigment epithelium.

CHALLENGES

Although stem cell therapy carries great potential for the treatment of retinal degeneration, its advancement to clinical translation faces multiple challenges. Among the most important are health and ethical issues associated with the nature of most stem cell types, such as risks of tumorigenesis associated with reprogramming and immunogenic responses. Risks associated with the surgery and microbiological safety can also be limiting factors for the use of stem cells. The type and number of cells needed for effective treatment, transplanted cell survival, and the functional outcomes remain questions of major importance for successful stem cell-based therapies in the clinic. Finally, production and delivery of clinical-grade stem cells involve unique regulatory and quality control requirements that need to be clearly established.

CONCLUDING REMARKS

Gene and cell therapies opened new doors in the treatment of currently incurable retinal degenerative disorders and promise to be the therapeutics of the future. To accelerate the advancement of this innovative field, expert groups have proposed key steps and recommendations. These recommendations address the most pressing needs for the development and delivery of effective treatments for retinal dystrophies in the next decade. The meeting of the National Eye Institute in collaboration with the National Institutes of Health Center for

Regenerative Medicine¹³² and the Monaciano Symposium¹³³ strongly recommended collaborative translational efforts, including the creation of international databases of correlative phenotype–genotype information, standardized protocols and outcome measures, common regulatory protocols, and technology transfer mechanisms.¹³⁴ There is a strong conviction that efficient partnerships between academia, industry, funding agencies, and policy makers are needed to translate laboratory discovery into the development of innovative gene and cell therapeutic strategies for the treatment of retinal degeneration.

ACKNOWLEDGMENTS

This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), Pierre et Marie Curie Université (UPMC), the Centre National de la Recherche Scientifique (CNRS), Foundation Fighting Blindness (FFB) (CD-CL-0808-0466-CHNO), and the French State program Investissements d'Avenir managed by the Agence Nationale de la Recherche (LABEX LIFESENSES: ANR-10-LABX-65).

AUTHOR DISCLOSURE

D.D. is a consultant for GenSight Biologics. O.G. and K.M. have no competing financial interests. J.-A.S. is a founder and consultant for Pixium Vision and GenSight Biologics, and a consultant for Sanofi-Fovea, Genesignal, and Vision Medicines.

REFERENCES

- Streilein JW. Ocular immune privilege: therapeutic opportunities from an experiment of nature. *Nat Rev Immunol* 2003;3:879–889.
- Caspi RR. A look at autoimmunity and inflammation in the eye. *J Clin Invest* 2010;120:3073–3083.
- Sahel JA, and Roska B. Gene therapy for blindness. *Annu Rev Neurosci* 2013;36:467–488.
- Reichel MB, Ali RR, Thrasher AJ, et al. Immune responses limit adenovirally mediated gene expression in the adult mouse eye. *Gene Ther* 1998;5:1038–1046.
- Kochanek S. High-capacity adenoviral vectors for gene transfer and somatic gene therapy. *Hum Gene Ther* 1999;10:2451–2459.
- Kreppel F, Luther TT, Semkova I, et al. Long-term transgene expression in the RPE after gene transfer with a high-capacity adenoviral vector. *Invest Ophthalmol Vis Sci* 2002;43:1965–1970.
- Puppo A, Cesi G, Marrocco E, et al. Retinal transduction profiles by high-capacity viral vectors. *Gene Ther* 2014;21:855–865.
- Von Seggern DJ, Aguilar E, Kinder K, et al. *In vivo* transduction of photoreceptors or ciliary body by intravitreal injection of pseudotyped adenoviral vectors. *Mol Ther* 2003;7:27–34.
- Cashman SM, McCullough L, and Kumar-Singh R. Improved retinal transduction *in vivo* and photoreceptor-specific transgene expression using adenovirus vectors with modified penton base. *Mol Ther* 2007;15:1640–1646.
- Sweigard JH, Cashman SM, and Kumar-Singh R. Adenovirus vectors targeting distinct cell types in the retina. *Invest Ophthalmol Vis Sci* 2010;51:2219–2228.
- Bennett J. Immune response following intraocular delivery of recombinant viral vectors. *Gene Ther* 2003;10:977–982.
- Pauwels K, Gijsbers R, Toelen J, et al. State-of-the-art lentiviral vectors for research use: risk assessment and biosafety recommendations. *Curr Gene Ther* 2009;9:459–474.
- Auricchio A, Kobinger G, Anand V, et al. Exchange of surface proteins impacts on viral vector cellular specificity and transduction characteristics: the retina as a model. *Hum Mol Genet* 2001;10:3075–3081.
- Duisit G, Conrath H, Saleun S, et al. Five recombinant simian immunodeficiency virus pseudotypes lead to exclusive transduction of retinal pigmented epithelium in rat. *Mol Ther* 2002;6:446–454.
- Kachi S, Binley K, Yokoi K, et al. Equine infectious anemia viral vector-mediated codelivery of endostatin and angiostatin driven by retinal pigmented epithelium-specific VMD2 promoter inhibits choroidal neovascularization. *Hum Gene Ther* 2009;20:31–39.

16. Bemelmans AP, Bonnel S, Houhou L, et al. Retinal cell type expression specificity of HIV-1-derived gene transfer vectors upon subretinal injection in the adult rat: influence of pseudotyping and promoter. *J Gene Med* 2005;7:1367–1374.
17. Balaggan KS, Binley K, Esapa M, et al. Stable and efficient intraocular gene transfer using pseudotyped EIAV lentiviral vectors. *J Gene Med* 2006;8:275–285.
18. Binley K, Widdowson P, Loader J, et al. Transduction of photoreceptors with equine infectious anemia virus lentiviral vectors: safety and bio-distribution of StarGen for Stargardt disease. *Invest Ophthalmol Vis Sci* 2013;54:4061–4071.
19. Colella P, and Auricchio A. AAV-mediated gene supply for treatment of degenerative and neo-vascular retinal diseases. *Curr Gene Ther* 2010;10:371–380.
20. Peeters L, Sanders NN, Braeckmans K, et al. Vitreous: a barrier to nonviral ocular gene therapy. *Invest Ophthalmol Vis Sci* 2005;46:3553–3561.
21. Greenberg KP, Geller SF, Schaffer DV, et al. Targeted transgene expression in Muller glia of normal and diseased retinas using lentiviral vectors. *Invest Ophthalmol Vis Sci* 2007;48:1844–1852.
22. Dalkara D, Kolstad KD, Caporale N, et al. Inner limiting membrane barriers to AAV-mediated retinal transduction from the vitreous. *Mol Ther* 2009;17:2096–2102.
23. Petrs-Silva H, Dinculescu A, Li Q, et al. High-efficiency transduction of the mouse retina by tyrosine-mutant AAV serotype vectors. *Mol Ther* 2009;17:463–471.
24. Kay CN, Ryals RC, Aslanidi GV, et al. Targeting photoreceptors via intravitreal delivery using novel, capsid-mutated AAV vectors. *PLoS One* 2013;8:e62097.
25. Dalkara D, Byrne LC, Klimczak RR, et al. *In vivo*-directed evolution of a new adeno-associated virus for therapeutic outer retinal gene delivery from the vitreous. *Sci Transl Med* 2013;5:189ra76.
26. Cronin T, Vandenbergh LH, Hantz P, et al. Efficient transduction and optogenetic stimulation of retinal bipolar cells by a synthetic adeno-associated virus capsid and promoter. *EMBO Mol Med* 2014;6:1175–1190.
27. Scalabrino ML, Boye SL, Fransen KM, et al. Intravitreal delivery of a novel AAV vector targets ON bipolar cells and restores visual function in a mouse model of complete congenital stationary night blindness. *Hum Mol Genet* 2015;24:6229–6239.
28. Vandenbergh LH, and Auricchio A. Novel adeno-associated viral vectors for retinal gene therapy. *Gene Ther* 2012;19:162–168.
29. Boyd RF, Sledge DG, Boye SL, et al. Photoreceptor-targeted gene delivery using intravitreally administered AAV vectors in dogs. *Gene Ther (in press)* doi: 10.1038/gt.2015.96.
30. Andrieu-Soler C, Bejjani RA, de Bizemont T, et al. Ocular gene therapy: a review of nonviral strategies. *Mol Vis* 2006;12:1334–1347.
31. Han Z, Conley SM, Makkia R, et al. Comparative analysis of DNA nanoparticles and AAVs for ocular gene delivery. *PLoS One* 2012;7:e52189.
32. Adijanto J, and Naash MI. Nanoparticle-based technologies for retinal gene therapy. *Eur J Pharm Biopharm* 2015;95:353–367.
33. Han Z, Conley SM, Makkia RS, et al. DNA nanoparticle-mediated ABCA4 delivery rescues Stargardt dystrophy in mice. *J Clin Invest* 2012; 122:3221–3226.
34. Han Z, Banworth MJ, Makkia R, et al. Genomic DNA nanoparticles rescue rhodopsin-associated retinitis pigmentosa phenotype. *FASEB J* 2015;29:2535–2544.
35. Konstan MW, Davis PB, Wagener JS, et al. Compacted DNA nanoparticles administered to the nasal mucosa of cystic fibrosis subjects are safe and demonstrate partial to complete cystic fibrosis transmembrane regulator reconstitution. *Hum Gene Ther* 2004;15:1255–1269.
36. den Hollander AI, Roepman R, Koenekoop RK, et al. Leber congenital amaurosis: genes, proteins and disease mechanisms. *Prog Retin Eye Res* 2008;27:391–419.
37. Morimura H, Fishman GA, Grover SA, et al. Mutations in the *RPE65* gene in patients with autosomal recessive retinitis pigmentosa or Leber congenital amaurosis. *Proc Natl Acad Sci U S A* 1998;95:3088–3093.
38. Li Y, Wang H, Peng J, et al. Mutation survey of known LCA genes and loci in the Saudi Arabian population. *Invest Ophthalmol Vis Sci* 2009;50: 1336–1343.
39. Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med* 2008; 358:2240–2248.
40. Hauswirth WW, Aleman TS, Kaushal S, et al. Treatment of Leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase I trial. *Hum Gene Ther* 2008;19:979–990.
41. Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med* 2008;358:2231–2239.
42. Cideciyan AV, Aleman TS, Boye SL, et al. Human gene therapy for RPE65 isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. *Proc Natl Acad Sci U S A* 2008;105:15112–15117.
43. Maguire AM, High KA, Auricchio A, et al. Age-dependent effects of *RPE65* gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. *Lancet* 2009;374:1597–1605.
44. Simonelli F, Maguire AM, Testa F, et al. Gene therapy for Leber's congenital amaurosis is safe and effective through 1.5 years after vector administration. *Mol Ther* 2010;18:643–650.
45. Ashtari M, Cyckowski LL, Monroe JF, et al. The human visual cortex responds to gene therapy-mediated recovery of retinal function. *J Clin Invest* 2011;121:2160–2168.
46. Jacobson SG, Cideciyan AV, Ratnakaram R, et al. Gene therapy for Leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. *Arch Ophthalmol* 2012;130:9–24.
47. Bennett J, Ashtari M, Wellman J, et al. AAV2 gene therapy readministration in three adults with congenital blindness. *Sci Transl Med* 2012; 4:120ra15.
48. Cideciyan AV, Aguirre GK, Jacobson SG, et al. Pseudo-fovea formation after gene therapy for RPE65-LCA. *Invest Ophthalmol Vis Sci* 2015; 56:526–537.
49. Ashtari M, Zhang H, Cook PA, et al. Plasticity of the human visual system after retinal gene therapy in patients with Leber's congenital amaurosis. *Sci Transl Med* 2015;7:296ra110.
50. Testa F, Maguire AM, Rossi S, et al. Three-year follow-up after unilateral subretinal delivery of adeno-associated virus in patients with Leber congenital amaurosis type 2. *Ophthalmology* 2013;120:1283–1291.
51. Cideciyan AV, Jacobson SG, Beltran WA, et al. Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. *Proc Natl Acad Sci U S A* 2013;110:E517–E525.
52. Jacobson SG, Cideciyan AV, Roman AJ, et al. Improvement and decline in vision with gene therapy in childhood blindness. *N Engl J Med* 2015;372:1920–1926.
53. Bainbridge JW, Mehat MS, Sundaram V, et al. Long-term effect of gene therapy on Leber's congenital amaurosis. *N Engl J Med* 2015;372: 1887–1897.
54. Testa F, Surace EM, Rossi S, et al. Evaluation of Italian patients with Leber congenital amaurosis due to AIPL1 mutations highlights the potential applicability of gene therapy. *Invest Ophthalmol Vis Sci* 2011;52:5618–5624.
55. MacLaren RE, Groppe M, Barnard AR, et al. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. *Lancet* 2014;383:1129–1137.
56. Scholl HP, and Sahel JA. Gene therapy arrives at the macula. *Lancet* 2014;383:1105–1107.
57. Koch SF, Tsai YT, Duong JK, et al. Halting progressive neurodegeneration in advanced retinitis pigmentosa. *J Clin Invest* 2015;125:3704–3713.
58. Boye SE, Boye SL, Pang J, et al. Functional and behavioral restoration of vision by gene therapy in the guanylate cyclase-1 (GC1) knockout mouse. *PLoS One* 2010;5:e11306.
59. Boye SL, Peshenko IV, Huang WC, et al. AAV-mediated gene therapy in the guanylate cyclase (RetGC1/RetGC2) double knockout mouse model of Leber congenital amaurosis. *Hum Gene Ther* 2013;24:189–202.

60. Manfredi A, Marrocco E, Puppo A, et al. Combined rod and cone transduction by adeno-associated virus 2/8. *Hum Gene Ther* 2013;24:982–992.
61. Burnight ER, Wiley LA, Drack AV, et al. *CEP290* gene transfer rescues Leber congenital amaurosis cellular phenotype. *Gene Ther* 2014;21:662–672.
62. Tan MH, Smith AJ, Pawlyk B, et al. Gene therapy for retinitis pigmentosa and Leber congenital amaurosis caused by defects in *AiPL1*: effective rescue of mouse models of partial and complete *AiPL1* deficiency using AAV2/2 and AAV2/8 vectors. *Hum Mol Genet* 2009;18:2099–2114.
63. Jacobson SG, Cideciyan AV, Aleman TS, et al. Human retinal disease from *AiPL1* gene mutations: foveal cone loss with minimal macular photoreceptors and rod function remaining. *Invest Ophthalmol Vis Sci* 2011;52:70–79.
64. Sun H, and Nathans J. ABCR: rod photoreceptor-specific ABC transporter responsible for Stargardt disease. *Methods Enzymol* 2000;315:879–897.
65. Kong J, Kim SR, Binley K, et al. Correction of the disease phenotype in the mouse model of Stargardt disease by lentiviral gene therapy. *Gene Ther* 2008;15:1311–1320.
66. Trapani I, Toriello E, de Simone S, et al. Improved dual AAV vectors with reduced expression of truncated proteins are safe and effective in the retina of a mouse model of Stargardt disease. *Hum Mol Genet* 2015;24:6811–6825.
67. Ou J, Vijayarathay C, Ziccardi L, et al. Synaptic pathology and therapeutic repair in adult retinosis mouse by AAV-RS1 transfer. *J Clin Invest* 2015;125:2891–2903.
68. Bonnet C, Augustin S, Ellouze S, et al. The optimized allotropic expression of *ND1* or *ND4* genes restores respiratory chain complex I activity in fibroblasts harboring mutations in these genes. *Biochim Biophys Acta* 2008;1783:1707–1717.
69. Ellouze S, Augustin S, Bouaita A, et al. Optimized allotropic expression of the human mitochondrial *ND4* prevents blindness in a rat model of mitochondrial dysfunction. *Am J Hum Genet* 2008;83:373–387.
70. Cwerman-Thibault H, Augustin S, Lechavue C, et al. Nuclear expression of mitochondrial *ND4* leads to the protein assembling in complex I and prevents optic atrophy and visual loss. *Mol Ther Methods Clin Dev* 2015;2:15003.
71. Chadderton N, Palfi A, Millington-Ward S, et al. Intravitreal delivery of AAV-NDI1 provides functional benefit in a murine model of Leber hereditary optic neuropathy. *Eur J Hum Genet* 2013;21:62–68.
72. Feuer WJ, Schiffman JC, Davis JL, et al. Gene therapy for Leber hereditary optic neuropathy: initial results. *Ophthalmology* (in press) doi: 10.1016/j.ophtha.2015.10.025.
73. Uretsky S, Sahel JA, Galy A, et al. A recombinant AAV2/2 carrying the wild-type *ND4* gene for the treatment of LHON: preliminary results of a first-in-man study and upcoming pivotal efficacy trials [abstract]. Presented at the 12th International Symposium on Ocular Pharmacology and Therapeutics (ISOPT Clinical), Berlin, Germany, July 9–12, 2015. Available from: http://events.eventact.com/windman/16720/ISOPT_Clinical_abstract_and_program_book.pdf
74. Folk JC, and Stone EM. Ranibizumab therapy for neovascular age-related macular degeneration. *N Engl J Med* 2010;363:1648–1655.
75. Leveillard T, Mohand-Said S, Lorentz O, et al. Identification and characterization of rod-derived cone viability factor. *Nat Genet* 2004;36:755–759.
76. Yang Y, Mohand-Said S, Danan A, et al. Functional cone rescue by RdCVF protein in a dominant model of retinitis pigmentosa. *Mol Ther* 2009;17:787–795.
77. Byrne LC, Dalkara D, Luna G, et al. Viral-mediated RdCVF and RdCVFL expression protects cone and rod photoreceptors in retinal degeneration. *J Clin Invest* 2015;125:105–116.
78. Leveillard T, and Sahel JA. Rod-derived cone viability factor for treating blinding diseases: from clinic to redox signaling. *Sci Transl Med* 2010;2:26ps16.
79. Ait-Ali N, Fridlich R, Millet-Puel G, et al. Rod-derived cone viability factor promotes cone survival by stimulating aerobic glycolysis. *Cell* 2015;161:817–832.
80. Komaromy AM, Rowland JS, Corr AT, et al. Transient photoreceptor deconstruction by CNTF enhances rAAV-mediated cone functional rescue in late stage CNGB3-achromatopsia. *Mol Ther* 2013;21:1131–1141.
81. MacLaren RE, Buch PK, Smith AJ, et al. CNTF gene transfer protects ganglion cells in rat retinae undergoing focal injury and branch vessel occlusion. *Exp Eye Res* 2006;83:1118–1127.
82. Lipinski DM, Barnard AR, Singh MS, et al. CNTF gene therapy confers lifelong neuroprotection in a mouse model of human retinitis pigmentosa. *Mol Ther* 2015;23:1308–1319.
83. Haurigot V, Villacampa P, Ribera A, et al. Long-term retinal PEDF overexpression prevents neovascularization in a murine adult model of retinopathy. *PLoS One* 2012;7:e41511.
84. Dalkara D, Kolstad KD, Guerin KI, et al. AAV mediated GDNF secretion from retinal glia slows down retinal degeneration in a rat model of retinitis pigmentosa. *Mol Ther* 2011;19:1602–1608.
85. Humayun MS, Dorn JD, da Cruz L, et al. Interim results from the international trial of Second Sight's visual prosthesis. *Ophthalmology* 2012;119:779–788.
86. Ho AC, Humayun MS, Dorn JD, et al. Long-term results from an epiretinal prosthesis to restore sight to the blind. *Ophthalmology* 2015;122:1547–1554.
87. Stingl K, Bartz-Schmidt KU, Besch D, et al. Artificial vision with wirelessly powered subretinal electronic implant alpha-IMS. *Proc Biol Sci* 2013;280:20130077.
88. Bi A, Cui J, Ma YP, et al. Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. *Neuron* 2006;50:23–33.
89. Lagali PS, Balya D, Awatramani GB, et al. Light-activated channels targeted to ON bipolar cells restore visual function in retinal degeneration. *Nat Neurosci* 2008;11:667–675.
90. Busskamp V, Duebel J, Balya D, et al. Genetic reactivation of cone photoreceptors restores visual responses in retinitis pigmentosa. *Science* 2010;329:413–417.
91. Mace E, Caplette R, Marre O, et al. Targeting channelrhodopsin-2 to ON-bipolar cells with vitreally administered AAV restores ON and OFF visual responses in blind mice. *Mol Ther* 2015;23:7–16.
92. Lin B, Koizumi A, Tanaka N, et al. Restoration of visual function in retinal degeneration mice by ectopic expression of melanopsin. *Proc Natl Acad Sci U S A* 2008;105:16009–16014.
93. Cehajic-Kapetanovic J, Eleftheriou C, Allen AE, et al. Restoration of vision with ectopic expression of human rod opsin. *Curr Biol* 2015;25:2111–2122.
94. Gaub BM, Berry MH, Holt AE, et al. Optogenetic vision restoration using rhodopsin for enhanced sensitivity. *Mol Ther* 2015;23:1562–1571.
95. van Wyk M, Pielecka-Fortuna J, Lowel S, et al. Restoring the ON switch in blind retinas: Opto-mGluR6, a next-generation, cell-tailored optogenetic tool. *PLoS Biol* 2015;13:e1002143.
96. Jacobson SG, Sumaroka A, Luo X, et al. Retinal optogenetic therapies: clinical criteria for candidacy. *Clin Genet* 2013;84:175–182.
97. Evans MJ, and Kaufman MH. Establishment in culture of pluripotent cells from mouse embryos. *Nature* 1981;292:154–156.
98. Takahashi K, and Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663–676.
99. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;131:861–872.
100. Alexander P, Thomson HA, Luff AJ, et al. Retinal pigment epithelium transplantation: concepts, challenges, and future prospects. *Eye (Lond)* 2015;29:992–1002.
101. Nazari H, Zhang L, Zhu D, et al. Stem cell based therapies for age-related macular degeneration: the promises and the challenges. *Prog Retin Eye Res* 2015;48:1–39.
102. Jayakody SA, Gonzalez-Cordero A, Ali RR, et al. Cellular strategies for retinal repair by

- photoreceptor replacement. *Prog Retin Eye Res* 2015;46:31–66.
103. Seiler MJ, and Aramant RB. Cell replacement and visual restoration by retinal sheet transplants. *Prog Retin Eye Res* 2012;31:661–687.
 104. Leach LL, and Clegg DO. Concise review: Making stem cells retinal: methods for deriving retinal pigment epithelium and implications for patients with ocular disease. *Stem Cells* 2015;33:2363–2373.
 105. Schwartz SD, Hubschman JP, Heilwell G, et al. Embryonic stem cell trials for macular degeneration: a preliminary report. *Lancet* 2012;379:713–720.
 106. Schwartz SD, Anglade E, Lanza R, et al. Stem cells in age-related macular degeneration and Stargardt's macular dystrophy—authors' reply. *Lancet* 2015;386:30.
 107. Trounson A, and McDonald C. Stem cell therapies in clinical trials: progress and challenges. *Cell Stem Cell* 2015;17:11–22.
 108. Stanzel BV, Liu Z, Somboonthanakij S, et al. Human RPE stem cells grown into polarized RPE monolayers on a polyester matrix are maintained after grafting into rabbit subretinal space. *Stem Cell Reports* 2014;2:64–77.
 109. Caras IW, Littman N, and Abo A. Proceedings: debilitating eye diseases. *Stem Cells Transl Med* 2014;3:1393–1397.
 110. Diniz B, Thomas P, Thomas B, et al. Subretinal implantation of retinal pigment epithelial cells derived from human embryonic stem cells: improved survival when implanted as a monolayer. *Invest Ophthalmol Vis Sci* 2013;54:5087–5096.
 111. RIKEN. Pilot clinical study into iPS cell therapy for eye disease starts in Japan [2013 July 30; accessed 2016 Jan 21]. Available from: http://www.riken.jp/en/pr/press/2013/20130730_1; Cyranoski D. Japanese woman is first recipient of next-generation stem cells [2014 Sept 12; accessed 216 Jan 21]. Available from: <http://www.nature.com/news/japanese-woman-is-first-recipient-of-nextgeneration-stem-cells-1.15915>
 112. Boroah S, Phillips MJ, Bilican B, et al. Using human induced pluripotent stem cells to treat retinal disease. *Prog Retin Eye Res* 2013;37:163–181.
 113. Chen FK, McLaren S, Edel M, et al. iPS cells for modelling and treatment of retinal diseases. *J Clin Med* 2014;3:1511–1541.
 114. McLaren RE, Pearson RA, MacNeil A, et al. Retinal repair by transplantation of photoreceptor precursors. *Nature* 2006;444:203–207.
 115. Barber AC, Hippert C, Duran Y, et al. Repair of the degenerate retina by photoreceptor transplantation. *Proc Natl Acad Sci U S A* 2013;110:354–359.
 116. Pearson RA, Barber AC, Rizzi M, et al. Restoration of vision after transplantation of photoreceptors. *Nature* 2012;485:99–103.
 117. Gonzalez-Cordero A, West EL, Pearson RA, et al. Photoreceptor precursors derived from three-dimensional embryonic stem cell cultures integrate and mature within adult degenerate retina. *Nat Biotechnol* 2013;31:741–747.
 118. Decembrini S, Koch U, Radtke F, et al. Derivation of traceable and transplantable photoreceptors from mouse embryonic stem cells. *Stem Cell Reports* 2014;2:853–865.
 119. Lamba DA, Gust J, and Reh TA. Transplantation of human embryonic stem cell-derived photoreceptors restores some visual function in Crx-deficient mice. *Cell Stem Cell* 2009;4:73–79.
 120. Eberle D, Schubert S, Postel K, et al. Increased integration of transplanted CD73-positive photoreceptor precursors into adult mouse retina. *Invest Ophthalmol Vis Sci* 2011;52:6462–6471.
 121. Lakowski J, Han YT, Pearson RA, et al. Effective transplantation of photoreceptor precursor cells selected via cell surface antigen expression. *Stem Cells* 2011;29:1391–1404.
 122. Reichman S, Terray A, Slembrouck A, et al. From confluent human iPS cells to self-forming neural retina and retinal pigmented epithelium. *Proc Natl Acad Sci U S A* 2014;111:8518–8523.
 123. Lakowski J, Gonzalez-Cordero A, West EL, et al. Transplantation of photoreceptor precursors isolated via a cell surface biomarker panel from embryonic stem cell-derived self-forming retina. *Stem Cells* 2015;33:2469–2482.
 124. Assawachananont J, Mandai M, Okamoto S, et al. Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. *Stem Cell Reports* 2014;2:662–674.
 125. Nakano T, Ando S, Takata N, et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell* 2012;10:771–785.
 126. Zhong X, Gutierrez C, Xue T, et al. Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs. *Nat Commun* 2014;5:4047.
 127. Bull ND, and Martin KR. Concise review: toward stem cell-based therapies for retinal neurodegenerative diseases. *Stem Cells* 2011;29:1170–1175.
 128. McGill TJ, Cottam B, Lu B, et al. Transplantation of human central nervous system stem cells—neuroprotection in retinal degeneration. *Eur J Neurosci* 2012;35:468–477.
 129. StemCells. Interim results show improvement in visual function and slowing of disease progression in phase I/II dry AMD trial [2014 June 19; accessed 2016 Jan 21]. Available from: http://investor.stemcellinc.com/phoenix.zhtml?c=86230&p=irol-newsArticle_print&ID=1941107
 130. Emerich DF, and Thanos CG. NT-501: an ophthalmic implant of polymer-encapsulated ciliary neurotrophic factor-producing cells. *Curr Opin Mol Ther* 2008;10:506–515.
 131. Sieving PA, Caruso RC, Tao W, et al. Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants. *Proc Natl Acad Sci U S A* 2006;103:3896–3901.
 132. Bharti K, Rao M, Hull SC, et al. Developing cellular therapies for retinal degenerative diseases. *Invest Ophthalmol Vis Sci* 2014;55:1191–1202.
 133. Thompson DA, Ali RR, Banin E, et al. Advancing therapeutic strategies for inherited retinal degeneration: recommendations from the Monaciano Symposium. *Invest Ophthalmol Vis Sci* 2015;56:918–931.
 134. O'Reilly M, Federoff HJ, Fong Y, et al. Gene therapy: charting a future course—summary of a National Institutes of Health Workshop, April 12, 2013. *Hum Gene Ther* 2014;25:488–497.

Received for publication November 10, 2015;
accepted after revision January 6, 2016.

Published online: January 11, 2016.