

Advances in gene therapy technologies to treat retinitis pigmentosa

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Abstract: Retinitis pigmentosa (RP) is a class of diseases that leads to progressive degeneration of the retina. Experimental approaches to gene therapy for the treatment of inherited retinal dystrophies have advanced in recent years, inclusive of the safe delivery of genes to the human retina. This review is focused on the development of gene therapy for RP using recombinant adenoassociated viral vectors, which show a positive safety record and have so far been successful in several clinical trials for congenital retinal disease. Gene therapy for RP is under development in a variety of animal models, and the results raise expectations of future clinical application. Nonetheless, the translation of such strategies to the bedside requires further understanding of the mutations and mechanisms that cause visual defects, as well as thorough examination of potential adverse effects.

Keywords: retinitis pigmentosa, photoreceptor, gene therapy, AAV

Introduction

The major causes of blindness are associated with malfunction and/or degeneration of retinal cells, which impairs the flow of visual information from the eye to the brain. Similar to other areas of the mammalian central nervous system, neurons of the retina are not replaced following degeneration. However, worldwide efforts aim to develop new therapies for preserving or improving retinal function. Such treatments are expected to slow the progression of degeneration, and if possible also to reverse its course. To help this endeavor, a number of animal models of retinal degeneration have recently led to a better understanding of mechanisms of retinal disease, and have consequently promoted the development of new therapeutic strategies.¹

Diseases of the retina display remarkable genetic and clinical heterogeneity.² Retinitis pigmentosa (RP) is the most common subtype of retinal degeneration, responsible for loss of vision in one in 4,000 people worldwide,³ one in 1,000 in the People's Republic of China,⁴ and one of 930 in India.⁵ RP can result from defects in any of more than 60 genes inherited as autosomal dominant (30%–40% of cases), autosomal recessive (50%–60%), or X-linked (5%–15%), and it can occur either alone or together with other systemic disorders. Notwithstanding, mutations in 30%–35% of RP patients cannot be identified.^{6–8}

Despite the heterogeneous genetic origin, RP patients display common clinical hallmarks, such as an abnormal, bone-spicule dark, punctuate pigmentation, typical of a thinned, degenerate retina; attenuated retinal vessels; dysfunction of the photoreceptors or the retinal pigment epithelium (RPE), marked by diminished electroretinogram (ERG) responses of both rods and cones, and progressive photoreceptor death. In most cases,

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rods are affected first, leading to night blindness, peripheral visual field loss leading to tunnel vision, and eventual total blindness. In a few cases, cones are affected first, causing loss of central vision. All RP conditions are progressive, but the speed and the pattern of deterioration of sight varies among patients. In fact, the same gene mutation can cause variable symptoms depending on the environment.⁶

Knowledge of underlying mechanisms of disease supports the design of optimal therapies. Such is the case with gene therapy, the aim of which is either to slow down or stop the progress of retinal degeneration in RP. Since the nature of the underlying mutation narrows down the range of treatment options, gene therapy for RP is discussed below according to the genetic classes of the disease.

Gene therapy for eye conditions

Currently, gene therapy represents the most promising therapeutic option for many inherited and acquired retinal diseases, and many preclinical and clinical assays have been done using gene therapy strategies. Recombinant adeno-associated virus (rAAV) is the most widely used vector for ocular gene delivery, because of its ability to transduce various retinal cell types in vivo efficiently,⁹ a result likely due to its small size relative to other viral vectors. Other advantages of AAV are the lack of pathogenicity and the ability to transduce both dividing and nondividing cells. While recombinant AAV vectors do not contain viral gene sequences, a neutralizing antibody response may be mounted against capsid proteins, which may impact the use of this vector in certain settings.¹⁰

The eye is arguably more amenable to gene therapy than other organs for several reasons: the structure and accessibility of the retina allow local, relatively noninvasive administration of the agent compared to other internal organs; treatment outcomes can be easily monitored both objectively and subjectively by noninvasive methods, such as electroretinography and optical coherence tomography, in addition to patient input; and the enclosed eye and the presence of the blood–retinal barrier prevent the unintentional systemic spread of vectors, and confer partial immune privilege status to the eye, thus limiting immune responses toward the transgene and the vector proteins.¹¹ Notwithstanding these features, rAAV vectors are subject to intense research to improve their efficacy in gene therapy.

Ideally, therapeutic gene modulation should be restricted to specific cell types. The penetration of AAV vectors can be limited by the site of injection for intravitreal injections allow targeting to the ganglion cell layer, while subretinal

injections target photoreceptors and RPE. In addition, distinct AAV-vector serotypes vary in both their targeting and transduction efficiency (Table 1).

Serotype tropism may also vary among distinct species. In canine models, various serotypes have been shown to transduce the outer retina, but recent work tends to favor especially serotypes 5 and 8 for the direct targeting of photoreceptors.^{12–23} In nonhuman primates, AAV2 shows good transduction in ganglion cells of the foveal area,²⁴ rod photoreceptors and RPE²⁵; AAV5 transduces primarily rods,²⁶ while primate cones may be targeted by AAV5 in combination with cone-specific promoters.²⁷ More recent work in primates showed effective transduction of photoreceptors with serotypes 1, 5, 8, and 9, with the latter showing particularly good transduction of cones.^{28–31} In several species, including primates, serotype 4 was shown to effectively target RPE.¹⁴

Tyrosine mutations in the capsid of AAV prevent vector ubiquitination and consequent degradation.³² New vectors have been developed in which the capsids of various AAV serotypes contain substitutions of phenylalanine for tyrosine residues. These provide for an increased efficiency of transduction, therefore reducing the amount of viral vector required for therapeutic effects, and consequently decreasing immune responses to the vector itself. Such substitutions result in increased penetration of the retina following intravitreal injections, which allows for the targeting of photoreceptors and RPE while avoiding the trauma of subretinal injections currently used to transduce these cell types.^{33,34}

The development of AAV vectors with cell-specific promoters helps in targeting the cell of interest. rAAV vectors containing a human *rpe65* promoter were used to induce RPE-specific expression in RPE65-deficient Briard dogs.

Table 1 Efficiency of transduction of different retinal cells following subretinal or intravitreal injection of different serotypes of AAV vectors in mice

Cell type/ injection site	RPE/ subretinal	Photoreceptor/ subretinal	Ganglion cell layer/intravitreal
Capsid			
AAV1	+++	–	–
AAV2	++	+	++
AAV4	++	–	–
AAV5	+	++	–
AAV8	+	+++	++
AAV9	–	+++	++

Notes: –, no transduction; + to +++, increasing transduction.
Abbreviations: AAV, adenoassociated virus; RPE, retinal pigment epithelium.

This was shown to be 10% stronger than the ubiquitous cytomegalovirus promoter, and was ineffective in older animals.¹⁷ More recently, RP guanosine triphosphatase (GTPase) regulator (RPGR) promoter region was characterized, and it may be useful in future RPE targeting.³⁵ Photoreceptors have been successfully targeted by both rhodopsin and rhodopsin-kinase promoters, with substantial activity in mice, dogs, and nonhuman primates,^{30,36,37} and a cone arrestin promoter has been used more recently for cone dystrophy.^{38,39} Promoters selective for retinal bipolar cells and ganglion cells have also been explored, mostly for optogenetic strategies of intervention through gene therapy, aimed at examining their use for recovery of visual function in patients at advanced stages of retinal degeneration.^{24,40}

Autosomal-recessive RP

In autosomal-recessive RP, the patient has two dysfunctional copies of the mutated gene. In this case, gene-replacement therapy constitutes a straightforward approach to treat both the defective genotype and phenotype. A key element of this strategy is that the therapy is directed at the retinal cells where the mutation or lack of the relevant gene causes the primary defect. Target cells are usually photoreceptors or the RPE.

One example of gene therapy for RP in animal models that mimics the human disorder is directed at mutations in the *MERTK* gene. Human receptor tyrosine kinase MER (MERTK) was originally cloned as a novel tyrosine kinase,⁴¹ expressed as a transmembrane protein with two fibronectin type III domains, two immunoglobulin-like C2-type domains, and one tyrosine-kinase domain.⁴¹ In addition to its potential onco-transforming ability, deletion of the *MERTK* gene was identified as the underlying defect in a classic rat model of RP: the Royal College of Surgeons (RCS) rat.⁴² Mutations in *MERTK* are responsible for a rare autosomal-recessive form of RP in humans.^{43–45}

Photoreceptors are exposed to intense levels of light that lead to the accumulation of photo-oxidized proteins and lipids, as well as free radicals, especially at the tips of the outer segments. Thus, photoreceptors have evolved to undergo constant outer-segment loss at their tips through RPE phagocytosis, together with renewal at their base via the cilium.^{46,47} Shed outer segments are digested in the RPE, from where important molecules are recycled to photoreceptors. Failure to regulate these functions properly can lead to the accumulation of debris in the interphotoreceptor space and retinal/RPE degeneration. MERTK located in the RPE mediates the association between these cells and the photoreceptor outer segments, and in *MERTK*-knockout mice, as well as in

the RCS rat, in which a truncated, nonfunctional MERTK fails to localize to the cell membrane, the normal ingestion by RPE cells of the shed tips of photoreceptor outer segments is impaired. The clearance of apoptotic cells by mononuclear phagocytes is also altered in *MERTK*-knockout mice.⁴⁸ These data are consistent with the function of MERTK in the cytoskeletal remodeling required for engulfment during phagocytosis.⁴⁹

Attempts to treat MERTK defects have thus far focused on gene transfer. It was reported that transfer of normal copies of the *MERTK* gene by adenoviral vectors into the subretinal space of RCS rats led to both histological and functional improvement 30 days after injection. Importantly, this included correction of RPE phagocytosis defects in areas near the injection site. However, the survival of photoreceptors appeared to be only transient.⁵⁰ Such transient phenotypic rescue with first-generation adenovirus vectors has been attributed to the immune response generated against viral gene products.⁵¹ Indeed, although the success of ocular gene therapy is credited in part to the relative immune-privileged status of the eye, a significant cellular immune response is known to be promoted by adenoviral proteins, which limits adenoviral-mediated transgene expression in the retina. Even though most of the adenoviral genome has been deleted in new generations of adenoviral vectors, this cellular immune response still represents a risk in the eye.⁵² Notwithstanding, these gene-transfer experiments with adenoviral vectors validated both that the disease phenotype is caused by mutations in *MERTK* and that the condition does respond to gene-replacement therapy.

In a later study, an AAV vector was used to transfer MERTK into the subretinal space of RCS rats, leading to restoration of phagocytic function, with a decrease in outer-segment debris. ERG analysis indicated transiently improved visual function and retinal morphology. The survival of photoreceptors was, however, prolonged for only 12 weeks, even in the presence of continued *MERTK* transgene expression.⁵³ One possible explanation is that once the degeneration machinery is triggered, it can be delayed but not prevented.⁵⁴ Since standard AAV-mediated transgene expression peaks at approximately 3–4 weeks posttreatment,⁵⁵ buildup of outer-segment debris may trigger photoreceptor degeneration before peak therapeutic activity. In addition, a single subretinal injection does not cover the entire area of the retina, and degenerating photoreceptors in distant parts of the retina may have a negative impact on photoreceptor survival in the treated area, thus further limiting the effects of the treatment.

Despite the incomplete photoreceptor rescue in early studies employing rAAV vectors, the results support gene therapy as a valid therapeutic approach, as long as faster-onset vectors are used. Indeed, when a lentiviral vector was used to transfer *MERTK*, functional improvements lasted up to 27 weeks, and photoreceptor-cell survival was prolonged for up to 30 weeks.⁵⁶

The most recent attempt to transfer *MERTK* via gene therapy took advantage of an AAV vector containing Y733F tyrosine-to-phenylalanine substitutions. This had been shown to provide rapid and efficient reporter-gene expression when injected subretinally into adult mouse eyes.³³ The rAAV-*MERTK* vector led to longer and more robust functional and morphological rescue than previous studies.⁵⁷

Autosomal-dominant RP

Only one mutated copy of the gene suffices to produce autosomal-dominant RP. Disease may be caused by reduction in the level of wild-type protein (haploinsufficiency), by a gain of a deleterious function (dominant negative effect), or by a combination of both. Dominant mutations may also lead to disease by causing the buildup of toxic proteins. While limited mechanistic insight has been gained from human patients, transgenic and targeted expression studies in animal models have been useful to distinguish among various types of dominant mutations. Because of the dominant nature of this class of disease, simple gene-replacement therapy is often insufficient to overcome the expression of the mutant allele, although haploinsufficiency disease may respond to gene-replacement therapy. Rather, gene therapy aimed at dominant diseases requires either suppression of the expression of the mutated allele or an increase in the expression of the wild-type allele, or both. Because there are often many disease-causing dominant mutations in a single gene, targeted gene elimination or repair for each separate mutation is problematic. An alternative approach would be to promote cell survival, to preserve affected retinal cells and slow the course of degeneration.

The most common mutations associated with autosomal dominant RP are in either the *RHO* or the *RDS/peripherin* gene, which account for approximately 25% and 10% of the cases, respectively.⁵⁸ Gene therapy approaches for each are presented below.

Rhodopsin RP

The first mutation described for RP was in the rhodopsin gene – *RHO*. Rhodopsin is the visual pigment in rod-photoreceptor cells, which subserves vision under dim

light conditions, and is involved in the essential first step of phototransduction. It consists of a protein moiety – an opsin – and a nonprotein moiety: the chromophore 11-*cis*-retinal. Opsin is a seven transmembrane domain-containing protein of the family of G-protein-coupled receptors, localized predominantly in the disk membranes of rod outer segments. Isomerization of 11-*cis*-retinal to all-*trans*-retinal upon absorption of a photon induces changes in opsin structure that promotes the activation of the G protein transducin, thus initiating the biochemical cascade known as phototransduction.⁵⁹ Rhodopsin accounts for >70% of the total rod outer-segment protein, and more than 120 mutations located in all three domains of rhodopsin – intradiskal, transmembrane, and cytoplasmic – are associated with RP. Almost all mutations lead to the production of aberrant protein.⁶⁰

The first rhodopsin mutation to be identified encoded a proline-to-histidine substitution at position 23 (P23H).^{61,62} P23H rhodopsin mutants are retained in the endoplasmic reticulum and are unable to associate with 11-*cis*-retinal.^{63–65} Unlike wild-type rhodopsin, mutant P23H is degraded by the ubiquitin–proteasome system,⁶⁶ but large quantities of unfolded, mutant protein accumulate as ubiquitinated P23H in the cytoplasm.⁶⁷ Similar to other dominant inherited neurodegenerative diseases, such as Parkinson's and amyotrophic lateral sclerosis, the formation of intracellular protein aggregates associate with cellular degeneration.⁶⁸ Mice heterozygous for *RHO* have normal retinal morphology and function, showing that the expression of just one functional rhodopsin allele is sufficient for vision. However, in many cases of RP, the dominant gain of function of misfolded rhodopsin induces degeneration of photoreceptors, and in such cases, mutations of one allele only lead to visual impairment. A plausible approach for the treatment of gain-of-function mutations is to enhance proteosomal degradation of misfolded rhodopsin, but to date there have been no reports of significant success in animal models. An alternative procedure might be through targeted ribonucleic acid (RNA)-based therapy to silence the mutant allele, while maintaining the expression of the wild-type allele. Gene-silencing therapies based on the selective destruction of a specific messenger RNA (mRNA) have been achieved with varying success using ribozymes, and more recently by RNA interference.^{69–71}

Ribozymes are self-cleaving RNA enzymes of approximately 30 nucleotides, naturally found in lower eukaryotes, viruses, and some bacteria. Their secondary structure is composed of three stems: the central stem is the catalytic domain responsible for the cleavage reaction, while the two flanking domains provide the antisense arms required for

mRNA binding that leads to sequence-specific cleavage.⁷² It was demonstrated that in vivo expression of an AAV-delivered ribozyme, designed to recognize and cleave the unique transcript produced by the P23H *RHO* transgene in rats, specifically reduced mRNA from the mutant allele, slowed the degeneration of photoreceptors, and led to functional preservation of the retina in the short term.⁷³ In later studies, it was shown that the continued expression of the ribozyme markedly slowed the rate of photoreceptor degeneration and preserved retinal function, as assessed by ERG, for at least 8 months in transgenic rats.^{74,75} These results were the first evidence that gene-silencing approaches for disease correction can be effective for long-term therapy in autosomal-dominant retinal degeneration. However, designing and testing such therapeutic reagents for the more than 120 different rhodopsin mutations presently known is not economically or technically viable. An alternative experiment was therefore designed to attack all rhodopsin mRNAs, wild-type and mutant, at once, with a silencing agent that recognizes a common target sequence while simultaneously delivering a replacement copy of *RHO*, the sequence of which is resistant to the action of the silencing agent.⁷⁶

More recently, small interfering RNA (siRNA) has emerged as more robust and efficient than ribozymes for silencing gene expression.⁷⁷ The silencing mechanism is based on ubiquitous cellular processes, which could arguably lead to more clinical success and public acceptance. RNA interference (RNAi) inhibits gene expression by degrading mRNA in a sequence-specific manner upon introduction of double-stranded RNA (dsRNA). This long dsRNA is cut into 21- to 23-mer active intermediates, the siRNAs, which are incorporated and unwound in the RNA-induced silencing complex (RISC).⁷⁸ When loaded with a single-stranded siRNA, RISC binds to the complementary sequence on the mRNA and cleaves the latter between nucleotides 10 and 11 of the siRNA,⁷⁹ thus initiating its degradation and inhibiting further gene expression.

In turn, the expression of functional RHO protein coded by *RHO* genes resistant to siRNA has been demonstrated through transgenesis, as well as by in vivo expression of the replacement gene delivered by rAAV in the presence of the targeting RNAi molecules.⁸⁰ Evidence of therapeutic benefit from AAV-delivered siRNA suppression and replacement therapies was obtained in transgenic P23H mice.^{76,81} These results were the first to show in vivo that the combination of suppression and replacement may treat dominantly inherited *RHO*-linked RP despite *RHO*-associated mutational heterogeneity.

RDS pattern dystrophy

Peripherin/RDS (retinal degeneration slow) is a transmembrane glycoprotein that, along with an associated protein, retinal outer-segment membrane, is localized to the rim region of outer-segment disks in rods and cones.^{82,83} Mice carrying a mutation in this gene (*RDS/RDS*) constitute one of the first and best-studied models of retinal degeneration since its phenotype was described in 1978.⁸⁴ This naturally occurring null mutant fails to form photoreceptor outer segments, whereas heterozygotes have a partial phenotype of short and disorganized outer segments,⁸⁵ suggesting that the protein level from one wild-type allele is not sufficient to maintain outer-segment structure and retinal function. Although these phenotypes implicate a requirement of RDS for correct outer-segment disk morphogenesis and maintenance of photoreceptor outer segments, its precise structural role is not yet completely understood.

Human *RDS*, cloned in 1991, encodes a putative 346-amino acid protein with 92% homology to the mouse protein.⁸⁶ In the same year, *RDS* mutations were identified in patients with autosomal dominant RP.⁸⁷ Thus far, over 90 human mutations in *RDS* have been identified, which result in a wide phenotypic spectrum of retinal dystrophies, related not only with RP but also with a variety of macular dystrophies, particularly pattern dystrophy. A common feature of these disorders is the loss of macular (central retinal) photoreceptors, a phenotype also seen throughout the *RDS*^{-/-} mouse retina. The genotypic and phenotypic heterogeneity make the extension of studies in animal models to humans uncertain. Currently, however, gene therapy appears to be the most promising approach for treating peripherin/RDS disease. Upon subretinal injection of an AAV vector containing *RDS*, homozygous null mice responded with increases in rhodopsin synthesis, correction of rod outer-segment formation, and restoration of visual function in the first 14 weeks following treatment.^{88,89} However, the treatment did not result in long-term preservation of photoreceptors,⁹⁰ demonstrating the critical importance of RDS in the integrity of the photoreceptor outer segment, and suggesting that the mutation-independent suppression and replacement strategies discussed above for *RHO* autosomal-dominant RP may be useful here as well.

Indeed, the concept of a double gene therapy strategy of siRNA-mediated suppression of RDS together with gene replacement through AAV vectors containing siRNA was validated with the demonstration of up to 50% reduction of RDS expression together with the simultaneous expression of a siRNA-resistant replacement transcript in the retina of mice in vivo.⁹¹

X-linked RP

X-linked RP (XLRP) is one of the most severe forms of RP, characterized by early onset and rapid progression of vision loss, accounting for 6%–20% of all RP cases. So far six loci of genetic defects have been mapped in XLRP (RP2 [MIM 312600], RP3 [MIM 312610], RP6 [MIM 312612], RP23 [MIM 300424], RP24 [MIM 300155], and RP34 [MIM 300605]), but only two genes were identified: *RP2* and *RPGR* or *RP3*.^{92,93} Studies in zebrafish provided insight into the cellular functions of both *RPGR/RP3* and *RP2*, due to the high degree of functional conservations between human genes and their orthologues.⁹⁴

Mutations in the *RPGR/RP3* gene account for 70% of XLRP and disease manifest in male patients with no male-to-male transmission of the phenotype. However, families with dominant inheritance and female carriers show disease symptoms of variable degree. *RPGR/RP3* product is essential for cell viability, and localizes in the connecting cilia and basal bodies of rod and cone photoreceptors, with a possible role in protein transport and microtubule organization. Approximately 60% of all XLRP cases are associated with a mutation hotspot in open reading frame in ORF15 of *RPGR/RP3*.^{95,96}

A potential gene therapy for *RPGR/RP3* XLRP was tested in two canine models through subretinal injection of rAAV serotype 5 coding human/*RP3* under human interphotoreceptor retinoid-binding protein or G-protein-coupled receptor kinase 1 promoters. Overall, the therapy was very effective, with preservation of photoreceptor nuclei and inner/outer segments. Both rod and cone photoreceptor functions were at higher levels in treated than in control eyes, thus providing proof of principle for translation to human treatment.²⁰

In turn, disease-causing mutations in the *RP2* gene account for approximately 15% of XLRP, and are spread more uniformly along the gene. Its product is believed to have a role in the trafficking of proteins to the plasma membrane and in maintaining Golgi cohesion. A majority of the mutations in *RP2* are localized in the cofactor C homologous domain, and are predicted to generate a truncated protein with disrupted localization.^{97,98} No gene therapy has yet been directed at this gene.

Neuroprotection in RP

Among the large number of mutations associated with RP, the common characteristic of the disease is the degeneration of rod and cone photoreceptors. Evidence both of endoplasmic reticulum stress, as well as execution of cell death through apoptosis, have been identified in photoreceptor-cell death. Accordingly, two gene therapy protocols have been designed

to interfere with these events, in an attempt to preserve the visual cells. Even a delay in degeneration may be important in a combination therapy, to allow the photoreceptors time to recover function as the expression of the replacement transgene increases.

Involvement of the endoplasmic reticulum stress response was identified among pathological events of RP.⁹⁹ Recently it was shown that rAAV-mediated overexpression of Bip, an endoplasmic reticulum chaperone, protected photoreceptors in the P23H *RHO* transgenic model of autosomal-dominant RP, and might be useful for other types of RP in which the mutation is related with the accumulation of misfolded proteins.¹⁰⁰ On the other hand, resistance to apoptosis was achieved with rAAV-mediated delivery of the *XIAP* gene in cultured human RPE cells,¹⁰¹ and its overexpression prolonged the effects of AAV-*PED6β* gene therapy.¹⁰² Also, preservation of photoreceptors in various models of RP was observed following rAAV delivery of various neurotrophic factors, such as glial cell line-derived neurotrophic factor, ciliary neurotrophic factor, brain-derived neurotrophic factor, and insulin-like growth factor, among others.

Future perspectives

AAV-vectored gene therapy is now common in mouse models of various human retinal diseases. A few therapies have made their way to clinical trials, but only one has advanced enough to pave the way towards clinical use, namely the ongoing Leber congenital amaurosis 2 trials.^{103–109} Even in this case, recent data unraveled a dissociation between functional recovery and prevention of photoreceptor-cell death.¹⁰⁸ Further aspects currently under investigation are related to immune responses to recombinant AAV vectors, especially upon readministration,^{105,110} as well as the relationship of age of onset with window of opportunity for gene therapy directed at distinct forms of Leber's congenital amaurosis.^{104,111}

As more studies advance, we expect further expansion of the field in the next 5–10 years. Clearly, retinal gene therapies will need to be tailored to each patient, so as to optimally address the state of the degeneration at the time of treatment. Single gene defects, if treated early, will benefit the most from direct replacement of therapeutic genes or correction/ablation of the offending gene. For many such disorders, however, patient numbers are so small that more general neuroprotective gene therapies are likely to be developed first, because they hold promise for preventing or delaying degeneration in a wide variety of retinal disorders.

For late-stage retinal dystrophies, in which photoreceptor-mediated vision is essentially absent, prosthetic retinas, cell-replacement therapies or gene-based modifications of inner retinal cells to become light receptors may be the most likely alternatives.¹¹² Stem and progenitor cells can be isolated from a number of sources, including embryonic tissue, adult brain, and even retina, prompting many researchers to investigate the potential for using these cells to generate retinal cells for transplantation.^{113,114} However, there are several obstacles to be overcome before these techniques can be applied, such as the poor yield of differentiation of exogenous stem cells and the complexity of the required synaptic connections between transplanted and endogenous retinal cells. Exploitation of cell replacement will require a deeper understanding of developmental biology and the identification of key regulators of the various cellular differentiation pathways.

In conclusion, the advances in the understanding of the genetics and pathophysiology of retinal disease have now established the fundamentals of new gene-based therapies for several disorders that have not responded to conventional treatments, and recombinant AAV vectors are at the center of this progress towards the cure of retinal degenerations.

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The authors report no other conflicts of interest in this work.

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