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Gene therapy for ocular diseases

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Abstract

The eye is an easily accessible, highly compartmentalised and immune-privileged organ that offers unique advantages as a gene therapy target. Significant advancements have been made in understanding the genetic pathogenesis of ocular diseases, and gene replacement and gene silencing have been implicated as potentially efficacious therapies. Recent improvements have been made in the safety and specificity of vector-based ocular gene transfer methods. Proof-of-concept for vector-based gene therapies has also been established in several experimental models of human ocular diseases. After nearly two decades of ocular gene therapy research, preliminary successes are now being reported in phase 1 clinical trials for the treatment of Leber congenital amaurosis. This review describes current developments and future prospects for ocular gene therapy. Novel methods are being developed to enhance the performance and regulation of recombinant adeno-associated virus- and lentivirus-mediated ocular gene transfer. Gene therapy prospects have advanced for a variety of retinal disorders, including retinitis pigmentosa, retinoschisis, Stargardt disease and age-related macular degeneration. Advances have also been made using experimental models for non-retinal diseases, such as uveitis and glaucoma. These methodological advancements are critical for the implementation of additional gene-based therapies for human ocular diseases in the near future.

The fundamental principles behind gene therapy were conceived more than half a century ago.¹ Nucleic acids were identified as the carriers of genetic information in the 1940s, and viral transduction was discovered less than a decade later. In the 1960s, scientists articulated the possible human health implications of using viral transduction to deliver therapeutic genes to patients. The discovery of reverse transcriptase and the advent of recombinant DNA technology enabled critical experimental advancements in the decades that followed. The first recombinant viral vectors were developed using the papilloma simian virus SV40, and it was demonstrated that retroviral vectors could correct the symptoms of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency and adenosine deaminase deficiency-severe combined immunodeficiency disease (SCID) in vitro. These accomplishments validated the prospects for clinically relevant gene therapy. When the first phase 1 clinical trials began in the early 1990s, it seemed that the age of human gene therapy had finally arrived.

Serious complications in early gene therapy clinical trials for ornithine transcarbamylase deficiency (OTCD)² and X-linked SCID³ raised ethical and safety concerns about the use of vector-based therapies in humans. Nevertheless, significant advancements have since been

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made in the field of human gene therapy, particularly for the treatment of ocular diseases. The eye is an easily accessible and immune privileged organ. Its compartmentalised anatomy permits localised delivery of viral vectors to specific ocular tissues, decreasing the doses needed for therapeutic effects and minimising the risks of systemic adverse effects. To date, more than 200 retinal disease loci and 160 ocular disease-relevant genes have been identified (Retinal Information Network: <http://www.sph.uth.tmc.edu/Retnet/>). Gene replacement and gene silencing have been consistently implicated as potentially efficacious therapies. Well-characterised animal models are available, the contralateral eye functions as an effective experimental control, and there are non-invasive methods for imaging the eye and measuring changes in ocular function. All of these features offer the eye unique advantages as a gene therapy target.

OCULAR GENE TRANSFER METHODS

Today, adeno-associated virus (AAV) vectors are among the most commonly used delivery systems for ocular gene therapy. AAV vectors do not integrate into the human genome and exist instead as extragenomic circular episomes, significantly decreasing the risk of insertional oncogenesis. They typically elicit minimal immune responses and allow for stable, long-term transgene expression in a variety of retinal cells, including photoreceptors, retinal pigment epithelium (RPE) cells, ganglion cells and Müller cells. These characteristics make AAV vectors particularly suited for applications in a variety of chronic ocular diseases.⁴ The earliest AAV vectors used genomic and protein capsid components that were both of serotype 2 (AAV2/2). Most recombinant AAV (rAAV) vectors today contain a genomic component of serotype 2, but capsids of many different AAV serotypes are commonly used. The capsid serotype determines the tropism and efficacy of the vector. For example, AAV2/2 and rAAV2/5 vectors transduce both the RPE and photoreceptors, but rAAV2/1, rAAV2/4 and rAAV4/4 vectors exclusively transduce the RPE. Deliberate serotype selection in the construction of rAAV vectors allows for enhanced specificity and performance.⁵

Inducible expression systems have also been developed for AAV vectors as an added control mechanism. Inducible systems driven by rapamycin,⁶ doxycycline⁷ or tetracycline⁸ have been reported for ocular applications in vivo. The use of a hypoxia responsive and aerobically silenced element (HRSE) upstream of a tissue-specific promoter can offer tight spatial and temporal control over transgene expression.⁹ This approach is particularly applicable for gene therapy targeting ocular neovascularisation.

Adenoviral (Ad) vectors, like AAV vectors, are non-integrating and transduce both dividing and non-dividing cells. However, they elicit robust cytotoxic T lymphocyte (CTL)-mediated immune responses that limit the duration of transgene expression.¹⁰ Helper-dependent Ad vectors can extend the duration of ocular expression from less than 3 months to up to 1 year.¹¹ Lentiviral vectors can induce stable, long-term transgene expression in anterior ocular structures, including the corneal endothelium and the trabecular meshwork, in addition to retinal tissues.¹² Because they are integrating vectors, justifiable concerns have been raised over the risk of insertional oncogenesis. Highly deleted,¹³ self-inactivating¹⁴ and non-integrating¹⁵ lentiviral vectors have been developed as safer alternatives.

There are also a variety of non-viral ocular gene transfer methods that have garnered interest because they are less limited by the size of the gene that may be transferred and because they do not raise the safety concerns that are associated with viral vectors.¹⁶ These methods include the use of DNA nanoparticles,¹⁷ the ϕ C31 integrase system,¹⁸ electroporation and lipofection,¹⁹ and though they have seen some tangible successes in ocular applications, they will not be discussed further in this review.

OCULAR GENE THERAPY IN EXPERIMENTAL MODELS

Table 1 summarises ocular gene therapy in experimental models.

Retinal and choroidal neovascularisation

Retinal and choroidal neovascularisation (CNV) play a pathogenic role in diabetic retinopathy, retinopathy of prematurity and age-related macular degeneration (AMD), which are among the most common causes of irreversible blindness worldwide.⁷⁵ Vascular endothelial growth factor (VEGF) is a potent angiogenic factor and an effective target for inhibiting retinal neovascularisation. Currently, anti-VEGF therapy is the only treatment approved for neovascular AMD. Soluble VEGF receptor (sFLT-1) is a specific inhibitor that binds and sequesters VEGF.⁷⁶ Pigment epithelium derived factor (PEDF), endostatin, angiostatin and tissue inhibitor of metalloproteinase-3 (TIMP3) are also demonstrated anti-angiogenic factors.⁷⁷

In the oxygen-induced ischaemic retinopathy mouse model, Ad and AAV vectors expressing *sFLT-1* reduce the number of neovascular endothelial cells by more than 50%.²⁰ In this model, rAAV2/1 mediated delivery of *sFLT-1*, endostatin, or *TIMP3*²² and HIV-mediated delivery of angiostatin²⁴ also effectively inhibit neovascularisation. In the spontaneously diabetic non-obese Torii (SDT) rat, AAV-sFLT-1 slows the progression of diabetic retinopathy.⁷⁸ Recently, we have also observed that AAV-sFLT-1 gene therapy ameliorates the AMD-like retinal lesions in the *Ccl2/Cx3cr1*-deficient mouse.²¹ Equine infectious anaemia virus (EIAV) lentiviral vectors carrying angiostatin or endostatin have been tested in a laser-induced murine model of CNV. Subretinal EIAV-endostatin and EIAV-angiostatin both cause reductions of approximately 50% in CNV area and 25% in vascular hyperpermeability with no vector-related morbidities for up to 1 year.²³

The efficacy of intravitreal and subretinal AAV-PEDF has been demonstrated for treating laser-induced CNV lesions in C57BL/6 mice.²⁵ A partially deleted adenoviral vector carrying *PEDF* (Ad-PEDF.11) also inhibits CNV in three different murine models²⁶ and a pig model²⁷ of CNV. A dose-related inflammatory response was observed for Ad-PEDF.11 in cynomolgus monkeys,²⁸ but the safety of this vector for the treatment of neovascular AMD has been demonstrated in a phase 1 clinical trial.⁷⁹

Photoreceptor, ganglion cell and optic nerve degeneration

Although there is currently no cure for retinitis pigmentosa (RP), well-characterised animal models and a developed understanding of the genetic basis of the disease allow gene therapy to be a potentially viable therapeutic strategy. The rhodopsin (RHO) promoted delivery of *Prph2* rescues both ultrastructure and function in differentiated photoreceptors of the *Rds* mouse model, which carries a mutation in *Prph2*.²⁹ It also increases the sensitivity of visually responsive neurons in the superior colliculus.³⁰ Although adult mice with advanced stages of photoreceptor degeneration still express transgenic *Prph2* and protect outer segments to some degree after treatment, greater improvements are generally seen in younger mice.³¹ These results emphasise the importance of early detection and prevention measures for the management of degenerative diseases. Although current mutation-specific and mutation-independent strategies for mutant RHO suppression are largely unable to rescue photoreceptor function, RNA interference (RNAi)-mediated mutant RHO suppression coupled with AAV mediated murine *Rho* replacement has been shown to provide significant functional improvements in a transgenic mouse expressing human Pro347Ser RHO.³³

In the *Rd* mouse, which carries a cGMP phosphodiesterase β -subunit (β PDE) mutation, the opsin-promoted expression of β PDE promotes photoreceptor survival and induces a twofold

increase in light sensitivity.³⁴ Lentiviral HIV-mediated β PDE delivery in newborn *Rd* mice prevents photoreceptor degeneration for up to 24 weeks.³⁵ The Royal College of Surgeons (RCS) rat is another well-characterised retinal degeneration model in which the RPE fails to phagocytise shed photoreceptor outer segments, and photoreceptor loss results. Mutated *Mertk*, a receptor tyrosine kinase primarily expressed in the RPE, contributes to the aberrant phenotype, and mutations in this gene are observed in some individuals with RP. Ad,⁴⁵ AAV⁴⁶ and HIV-1 lentivirus⁴⁷ -mediated *Mertk* delivery effectively restores RPE function and slows photoreceptor degeneration in juvenile RCS rats for up to 4 weeks, 9 weeks and 7 months, respectively.

Leber congenital amaurosis (LCA), an autosomal recessive disorder that affects both rods and cones, has been linked to at least 14 genes. Several have been demonstrated as potentially efficacious gene therapy targets. One of the earliest genes to be identified is *GUCY2D*, which encodes photoreceptor-specific guanylate cyclase-1 (RETGC1). Mutations in *GUCY2D* are linked to LCA type 1 and implicated in up to 20% of LCA cases.⁸⁰ In *GUCY1*B* chickens, which carry null mutations in *GUCY2D*, lentiviral-mediated gene transfer partially restores electroretinogram (ERG) responses and slows retinal degeneration.⁴⁸ Gene therapy targeting aryl hydrocarbon receptor-interacting protein-like 1 (*AIR1*) and RP GTPase regulator interacting protein (*RPGRIP*) has also been shown to be successful.^{49,50}

RPE65 is a 65-kDa membrane-associated phototransduction protein responsible for converting all-*trans* retinyl esters to 11-*cis* retinol in the RPE.⁸¹ *RPE65* mutations result in RHO depletion, all-*trans* retinal accumulation and photoreceptor dysfunction and are present in 6.0% of LCA cases.⁸² In the *Rd12* mouse, which has a recessive nonsense *Rpe65* mutation, rAAV2/5-mediated gene replacement restores rod photoreceptor function and vision-dependent behaviour and maintains stable retinal morphology for at least 7 months.⁵¹ Lentiviral vectors selectively transduce the RPE and protect cone structure and function in *Rpe65*^{-/-} mice.⁵² AAV-RPE65⁵³ and rAAV2/4-RPE65⁵⁴ stably and effectively restore rod and cone function in *RPE65*-deficient dogs. Safety studies have shown that AAV-RPE65 is also well tolerated in Briard dogs⁸³ and cynomolgus monkeys.⁵⁵ Experiments in murine⁵⁶ and canine⁵⁴ models have shown that *RPE65* gene therapy may be more effective during early disease stages, when the photoreceptor layer is still well preserved. Early treatment may allow for better rescue of cone function in particular.⁸⁴

Although gene therapy prospects for retinal degeneration have been most thoroughly explored for LCA and RP, there are additional degenerative diseases for which gene therapy has shown promise. Examples include AAV-mediated guanine nucleotide α -transducin (*GNAT2*) gene replacement for achromatopsia,⁵⁷ AAV⁵⁸ and EIAV⁵⁹ -mediated *ABCA4* delivery for Stargardt disease, and AAV-mediated retinoschisin (*RS1*) gene replacement for X linked juvenile retinoschisis.⁶⁰

Apoptosis has been shown to be the final common pathway for photoreceptor cell death in the *Rd*, *Rds* and *Rho* knockout mice.⁸⁵ Apoptosis pathway disruption and neurotrophic factor supplementation have both been explored as therapeutic strategies. In the *Rd* mouse, the rhodopsin-promoted delivery of the anti-apoptotic gene *Bcl-2* inhibits photoreceptor degeneration for up to 6 weeks.³⁶ simian immunodeficiency virus (SIV) lentivirus-mediated delivery of the neurotrophic factor *PDGF* has also been shown to have anti-apoptotic effects and preserve electrical function in RCS rats.³⁷ In transgenic rats expressing mutant *RHO*, AAV-mediated fibroblast growth factor (*FGF-2*) delivery significantly slows the rate of photoreceptor degeneration.³⁸ When simultaneously treated with both SIV-PEDF and SIV-FGF-2, RCS rats and *Rds* mice saw synergistically and significantly amplified therapeutic effects.³⁹ In the *Rds* mouse, systemic but not intraocular delivery of the neurotrophic factor

erythropoietin (*Epo*) rescues photoreceptor morphology.⁸⁶ Gene therapy using the ciliary neurotrophic factor (*CNTF*) promotes photoreceptor survival in the *Rds*,⁴¹ *Rd42* and *Rho* knockout⁴³ mice. Despite these results, Schlichtenbrede *et al* showed that AAV-CNTF may diminish retinal function in both *Rds* and wild-type mice.⁴⁴

Glaucoma is characterised by retinal ganglion cell (RGC) loss, which leads to optic nerve atrophy, and neurotrophic factors such as brain-derived neurotrophic factor (BDNF), CNTF and glial cell derived neurotrophic factor (GDNF) have been shown to have protective effects.¹ In a rat model of glaucoma, AAV-BDNF significantly increases RGC survival.⁶⁴ Using the optic nerve transection model, lentiviral-mediated *CNTF* therapy preserves axotomised RGCs for 3 weeks.⁶⁵ Recent experiments in cynomolgus monkeys have also shown that feline immunodeficiency virus (FIV) lentiviral vectors may be used to transduce the aqueous humour outflow pathway, allowing for potential gene therapies targeting intraocular pressure.⁸⁸ Due to inflammatory responses in the anterior chamber, non-viral gene transfer should be considered.⁸⁹ *GDNF* plasmid DNA delivered to glial cells via electroporation significantly increases RGC survival and decreases the expression of apoptotic factors.⁶⁶

Other ocular diseases

Gene therapy has also been investigated for experimental autoimmune uveitis (EAU). The Th2 cytokine interleukin (IL)-10 has demonstrated anti-inflammatory effects, and adenoviral-mediated *IL-10* gene transfer in mouse and rat models affords significant reductions in EAU.⁶⁷ The IL-1 receptor agonist (IL-1Ra) exerts anti-inflammatory effects by blocking IL-1 signalling. AAV-IL-1Ra significantly ameliorates experimental uveitis for up to 100 days in rabbits.⁷⁰

Retinoblastoma is the most common primary ocular malignancy in children, and gene therapy has been extensively investigated as a potential treatment. Interferon- β (IFN- β) is used for several malignancies, but systemic administration often causes toxicity. Local ocular delivery of AAV-IFN- β by intravitreal injection is well tolerated and effectively inhibits tumour growth in a rat model of retinoblastoma.⁷¹ Suicide gene therapy, using adenoviral-mediated delivery of the herpes simplex thymidine kinase gene (AdV-TK) followed by ganciclovir administration, inhibits DNA synthesis and has demonstrated tumour suppressive effects in other malignancies.^{90–92} In mice, AdV-TK exclusively transduces retinoblastoma cells and inhibits tumour growth when coupled with intraocular injections of ganciclovir.⁷²

Gene therapy has also been explored as a potential therapy for red-green colour blindness,⁷³ corneal neovascularisation,⁷⁴ a variety of other corneal diseases, including allograft rejection, postexcimer laser scarring and herpes simplex keratitis,⁹³ uveal melanoma⁹⁴ and optic nerve diseases, including optic nerve trauma, optic neuritis and Leber's hereditary optic neuropathy.⁹⁵

OCULAR GENE THERAPY CLINICAL TRIALS

Neovascular AMD

Campochiaro *et al* performed a phase 1 clinical trial to assess the safety of intravitreal injections of AdPEDF.11 in 28 patients with neovascular AMD.⁷⁹ At all tested doses, no serious adverse events were observed. Mild ocular inflammation and transient increases in intraocular pressure controlled with topical medication were reported in 25% and 21% of patients, respectively. Because placebo controls were not included, assessments of efficacy could not be performed. Nevertheless, treatment did not have any negative effects on visual acuity, and decreases in the size and prominence of CNV lesions were found in the high-

dose treatment group by funduscopy and fluorescein angiography. The low-dose group showed declines in visual acuity and increases in lesion size 6 months after injection. Gene therapy using AdPEDF.11 at doses of at least 1×10^8 particle units merits further exploration as a treatment for human neovascular AMD. Patients are also currently being recruited for a phase 1 clinical trial investigating the safety and tolerability of AAV2-sFLT01⁹⁶ gene therapy (<http://clinicaltrials.gov/>).

LCA

In light of the successes of *RPE65* gene therapy in experimental models of LCA, three independent groups initiated phase 1 human clinical trials.^{97–99} Each group enrolled three patients and assessed the effects of a single subretinal injection of AAV2/2-RPE65. Bainbridge *et al* showed no significant adverse events and no significant changes in ERG response, visual acuity or peripheral visual field after 6 months.⁹⁷ One patient showed significant improvements in dark-adapted function and visual mobility, though this patient had less severe retinal disease at baseline. Cideciyan *et al* reported statistically significant improvements in visual sensitivity mediated by both rod and cone photoreceptor function 30 days post-treatment that were sustained after 1 year.^{98,100} Maguire *et al* described significantly improved pupillary light reflexes and self-reported improvements in dim light vision.⁹⁹ No vector-mediated adverse events were observed, though one patient developed a full thickness macular hole, and functional improvements persisted 1.5 years post-treatment.¹⁰¹ In a dose-escalation study involving 12 patients and performed by the same group, safety and efficacy were sustained for at least 2 years post-treatment.¹⁰² Viral vectors were transiently detected in the tears and blood after surgery, but no significant immune responses were reported. Improvements in light sensitivity also correlated closely with age and baseline sensitivity, suggesting that younger individuals with better structural preservation are more likely to respond positively to treatment.

Although each group used AAV-mediated *RPE65* gene transfer, there are important technical differences between the trials. The vectors utilised different promoters and were prepared at different manufacturing facilities. The injections were administered at different volumes to different locations in the retina, with or without corticosteroids. Patients also varied in age and baseline visual function. All of these variations make it challenging to integrate the results from patients in different studies in a meaningful way. Further investigation is still needed to determine an optimal vector design, manufacturing strategy and administration protocol. Nevertheless, the clinical trials have consistently suggested that treatment age and retinal integrity are strong predictors of patient outcomes, in agreement with previously reported animal studies. *RPE65* gene therapy for LCA has been one of the most successful examples of gene therapy for ocular diseases to date. Several additional *RPE65* gene therapy clinical trials are currently in progress (<http://clinicaltrials.gov/>).

Retinoblastoma

Chávez-Barrios *et al* reported the results of a phase 1 clinical trial investigating the safety and efficacy of intravitreal injections of AdV-TK followed by systemic ganciclovir for the treatment of bilateral retinoblastoma with vitreous tumour seeding.¹⁰³ Eight paediatric patients who had failed systemic chemotherapy, external-beam radiotherapy and local treatments, such as cryotherapy and laser therapy, were enrolled. A patient treated with one injection at the lowest dose showed no gene-therapy related toxicity, but most patients treated with higher doses showed mild to moderate uveitis. In the two patients treated at the highest doses, corneal oedema and increased intraocular pressure developed, followed by significant vision loss. Tumour regression was observed at higher doses, though all ultimately required enucleation, indicating that suicide gene therapy should not be used alone but might be considered as an adjuvant to standard therapies.

Transgene expression was not evaluated, and though no retinal damage was clinically observed, the extent of toxic *TK* expression in non-retinoblastoma cells remains uncharacterised. Novel methods to enhance tumour cell specificity have been explored. A conditionally replicating adenovirus (CRAd) coupled with human telomerase reverse transcriptase (hTERT), a tumour-specific promoter, has been explored as potential a delivery system for the gene encoding TK.¹⁰⁴ This vector induces exclusive transgene expression in human retinoblastoma cells without detectable expression levels in the RPE, opening the possibility of significantly enhancing the safety of suicide gene therapy/ganciclovir treatment for human retinoblastoma.

CONCLUSIONS

Notable progress has been made in understanding the genetic pathogenesis of ocular diseases and improving the safety and specificity of vector-based ocular gene transfer methods. These technical developments have been critical to the gene therapy successes achieved in experimental models of both retinal and non-retinal ocular diseases. The majority of experiments have been performed in mice, however, and more work is needed in animals with eyes that more closely resemble those of humans. Preliminary successes have been reported in phase 1 clinical trials utilising *RPE65* gene therapy for LCA and *PEDF* gene therapy for AMD. Given the success of anti-VEGF therapy using monoclonal antibody, chimeric protein and oligonucleotide approaches,⁷⁷ and the positive results seen for anti-VEGF gene therapy in animal models, the upcoming phase 1 clinical trial for AAV2-sFLT01 gene therapy holds considerable promise. With the methodological advancements of the last decade and the establishment of proof-of-concept for gene therapy approaches targeting a variety of previously uncharted conditions, gene therapy may indeed be an essential treatment strategy for ocular diseases in the near future.

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Table 1

Gene therapy in experimental models of ocular diseases

Gene	Vector	Delivery	Model	Outcome	Reference
Neovascularisation					
<i>sFlt-1</i>	Ad- or AAV-CMV-sFLT-1	Intravitreal	Oxygen-induced ischaemic retinopathy mouse	Reduced number of neovascular endothelial cells >50%	20
<i>sFlt-1</i>	AAV-CBA-sFLT-1	Subretinal	<i>Ccl2/Cx3cr1</i> DKO mouse	Reduced retinal lesions	21
Endostatin	rAAV2/1-CMV-endostatin	Subretinal	Oxygen-induced ischaemic retinopathy mouse	Decreased neovascular tufts and vessel leakage, inhibited inner retinal neovascularisation	22
Endostatin	ElAV-endostatin	Subretinal	Laser-induced CNV mouse	Reduced CNV area by 59.9%, hyperpermeability by 25.6%, decreased apoptosis	23
Angiostatin	HIV-angiostatin	Intravitreal	Oxygen-induced ischaemic retinopathy mouse	Reduced neovascular nuclei by 68–87% in 90% of animals	24
Angiostatin	ElAV-angiostatin	Subretinal	Laser-induced CNV mouse	Reduced CNV area by 50.0%, hyperpermeability by 23.9%	24
<i>TIMP3</i>	rAAV2/1-CMV-TIMP3	Subretinal	Oxygen-induced ischaemic retinopathy mouse	Decreased neovascular tufts and vessel leakage, inhibited inner retinal neovascularisation	22
<i>PEDF</i>	rAAV2/1-CMV-PEDF	Subretinal	Oxygen-induced ischaemic retinopathy mouse	Decreased neovascular tufts and vessel leakage, inhibited inner retinal neovascularisation	22
<i>PEDF</i>	AAV-CBA-PEDF	Subretinal	Laser-induced CNV mouse	Inhibited laser-induced CNV	25
<i>PEDF</i>	AdPEDF.11	Subretinal	3 murine CNV models	Inhibited laser and ischaemia-induced neovascularisation, decreased CNV lesion area	26
<i>PEDF</i>	AdPEDF.11	Periocular	Laser-induced CNV pig	Inhibited laser-induced CNV	27
<i>PEDF</i>	AdPEDF.11	Intravitreal	Cynomolgus monkey	Dose-related inflammatory response	28
Retinitis pigmentosa					
<i>Prlp2</i>	AAV-Rho-Prph2	Subretinal	<i>Prlp2^{Rd2/Rd2}</i> mouse	Long-term photoreceptor ultrastructure preservation, ERG rescue, improved central superior colliculus signalling, more improvement in younger subjects	29 – 32
<i>Rho</i>	rAAV2/5-RHO	Subretinal	Human Pro347Ser RHO transgenic mouse	RNAi-mediated RHO suppression coupled with gene replacement improved ERG	33
<i>BPDE</i>	AAV-opsin-BPDE	Intravitreal	<i>Rd</i> mouse	Improved photoreceptor survival, increased light sensitivity twofold	34
<i>BPDE</i>	HIV-CMV-BPDE	Subretinal	<i>Rd</i> mouse	Prevented photoreceptor degeneration for up to 24 weeks	35

Gene	Vector	Delivery	Model	Outcome	Reference
<i>Bcl2</i>	Ad-2.5HRP-Bcl2	Subretinal	<i>Rd</i> mouse	Rescued photoreceptors for up to 6 weeks, not as effective as Ad-CMV-BPDE	36
<i>PEDF</i>	SIV-PEDF	Subretinal	RCS rat	Preserved light sensitivity, prevented photoreceptor loss	37
<i>FGF-2</i>	AAV-CMV-FGF-2	Subretinal	TgN S334ter-4 transgenic rat	Enhanced photoreceptor survival, did not significantly improve ERG	38
<i>PEDF</i> and <i>FGF-2</i>	SIV-PEDF and SIV-FGF-2	Subretinal	RCS rat	Synergistic protective effects in photoreceptors over SIV-PEDF or SIV-FGF-2 alone	39
<i>Epo</i>	AAV-CMV-Epo	Intramuscular	<i>Prlpl2^{Rd/Rd}</i> mouse	Systemic but not intraocular delivery prevented photoreceptor death, no effect in rd10 mouse	40
<i>CNTF</i>	Ad-CMV-CNTF	Intravitreal	<i>Rds</i> mouse	Prevented photoreceptor degeneration, improved ERG response	41
<i>CNTF</i>	Ad-CMV-CNTF	Intravitreal	<i>Rd</i> mouse	Preserved photoreceptors for at least 18 days	42
<i>CNTF</i>	AAV-CMV-CNTF-GFP	Subretinal	Rhodopsin KO mouse	Preserved photoreceptors for up to 3 months	43
<i>CNTF</i>	AAV-CMV-CNTF-GFP	Subretinal	<i>Prlpl2^{Rd/Rd}</i> mouse	Prevented photoreceptor loss, caused visual function loss, 50% reduction in b-wave amplitude	44
<i>Mertk</i>	Ad-CMV-Mertk	Subretinal	RCS rat	Increased light sensitivity, preserved photoreceptor outer segment for 4 weeks	45
<i>Mertk</i>	AAV-CMV-Mertk	Subretinal	RCS rat	Preserved photoreceptor survival and function for up to 9 weeks, restored RPE function	46
<i>Mertk</i>	HIV-SFFV-Mertk	Subretinal	RCS rat	Slowed photoreceptor cell loss, preserved function for up to 7 months	47
Leber congenital amaurosis					
<i>GUCY2D</i>	pTYF-EF1 α -GC1-IRES-eGFP	Embryonic	GUCY1*B chicken	Slowed retinal degeneration, partially restored ERG response	48
<i>AIP1</i>	rAAV2/5-RK-Aip1	Subretinal	<i>Aip1^{-/-}</i> and <i>Aip1^{hypo}</i> mouse	Restored PDE synthesis, enhanced rod and cone survival	49
<i>RPGRIP</i>	AAV-opsin-RPGRIP	Subretinal	<i>RPGRIP^{-/-}</i> mouse	Corrected RPGR localisation, protected photoreceptors, improved ERG	50
<i>RPE65</i>	rAAV2/5-CBA-hRPE65	Subretinal	<i>Rd12</i> mouse	Improved rhodopsin levels, corrected ERG for 7 months, early treatment more effective	51
<i>RPE65</i>	HIV-CMV-Rpe65	Subretinal	<i>Rpe65^{-/-}</i> mouse	Corrected ERG, prevented cone degeneration for 4 months	52

Gene	Vector	Delivery	Model	Outcome	Reference
<i>RPE65</i>	AAV-RPE65	Subretinal	<i>RPE65</i> ^{-/-} dog	Partially restored ERG, improved pupillary response, visual function for 3 years, well tolerated	53
<i>RPE65</i>	rAAV2/4-RPE65	Subretinal	<i>RPE65</i> ^{-/-} dog	Restored rod and cone function for 8–11 months, not effective in older dogs	54
<i>RPE65</i>	AAV-RPE65	Subretinal	Cynomolgus monkey	No systemic toxicity, mild ocular inflammation, no vector sequences in optic nerve or brain	55
<i>RPE65</i>	rAAV2/1-CMV-hRPE65	Subretinal	<i>Rpe65</i> ^{-/-} mouse	Therapy effective in advanced disease stages if photoreceptor structure well preserved	56
Achromatopsia					
GNAT2	rAAV2/5-PR2.1-Gnat2	Subretinal	<i>Gnat2</i> ^{oph3} mouse	Rescued cone-mediated ERG response and visual acuity	57
Stargardt disease					
<i>ABCA4</i>	rAAV2/5-CMV-ABCA4	Subretinal	<i>Abca4</i> ^{-/-} mouse	Corrected lipofuscin levels, improved RPE morphology and retinal function	58
<i>ABCA4</i>	ElAV-CMV-ABCA4	Subretinal	<i>Abca4</i> ^{-/-} mouse	Reduced A2E accumulation	59
Retinosis					
<i>Rsl</i>	AAV-CMV-Rslh	Intravitreal	<i>Rsl</i> KO mouse	Rescued retinal morphology, ERG, restored synaptic connections in photoreceptors	60 61
<i>Rsl</i>	rAAV2/8-Rslh	Intravitreal	<i>Rsl</i> KO mouse	Decreased schisis cavities, improved ERG	62
<i>Rsl</i>	rAAV2/5-opsin-Rslh	Subretinal	<i>Rsl</i> KO mouse	Slowed photoreceptor degeneration, improvements observed during late disease stages	63
Glaucoma					
<i>BDNF</i>	AAV-BDNF	Intravitreal	Laser-induced elevated IOP rat model	Promoted retinal ganglion cell survival	64
<i>CNTF</i>	SIN-PGK-CNTF	Intravitreal	Optic nerve transection rat model	Rescued axotomised retinal ganglion cells for up to 3 weeks	65
<i>GDNF</i>	-	Electroporation	Optic nerve transection rat model	Increased retinal ganglion cell survival, decreased expression of apoptotic factors	66
Uveitis					
<i>IL-10</i>	Ad-vIL-10	Retro-orbital sinus	IRBP-treated mouse or S-Ag-treated Lewis rat	Protected against IRBP- or S-Ag-induced EAU	67
<i>IL-10</i>	AAV-IL-10	Subretinal	IRBP treated mouse	Protected against IRBP-induced EAU	68
<i>IL-10</i>	AAV-tetON-vIL-10	Intravitreal	S-Ag treated Lewis rat	Protected against S-Ag-induced EAU, rAAV2/5-tetON-vIL-10 ineffective	69
<i>IL-1Ra</i>	AAV-CMV-IL-1Ra	Intravitreal	IL-1a treated rabbit	Reduced inflammation and leucocyte	70

Gene	Vector	Delivery	Model	Outcome	Reference
Retinoblastoma				infiltration	
<i>IFN-β</i>	AAV-IFN-β	Intravitreal	Orthotopic xenograft rat	Conferred significant anti-tumour effects, little or no systemic toxicity	71
<i>TK</i>	Adv-TK and ganciclovir	Intravitreal	<i>Rag-2</i> KO immunodeficient mouse	Completely ablated tumour in 70%, extended progression-free survival	72
Red-green colour blindness					
L-opsin	rAAV2/5-L-opsin	Subretinal	L-opsin-deficient adult squirrel monkey	Restored trichromatic colour vision behaviour	73
Corneal neovascularisation					
<i>GABP</i>	Lipoplexed plasmid DNA	Subconjunctival	Deliberate corneal injury mouse model	Delayed corneal neovascularisation for up to 2 weeks	74

AAV, adeno-associated virus; ABCA4, ATP-binding cassette, sub-family A (ABC1), member 4; APL1, aryl hydrocarbon receptor-interacting protein-like 1; A2E, (2-[2,6-dimethyl 1-8-(2,6-dimethyl 1-1-cyclohexen-1-yl)-1E,3E,5E,7E-octatetraenyl]-1-(2-hydroxyethyl)-4-[4-methyl 1-6(2,6,6-trimethyl-1-cyclohexen-1-yl)-1 E,3E,5E,7E-hexatrienyl]-pyridinium)); BDNF, brain-derived neurotrophic factor; BPDE, cGMP phosphodiesterase β-subunit; CBA, chicken β-actin; CMV, cytomegalovirus; CNTF, ciliary neurotrophic factor; CNV, choroidal neovascularisation ; DKO, double knockout; EAU, experimental autoimmune uveitis ; EF1α, elongation factor 1α; EIAV, equine infectious anaemia virus; EPO, erythropoietin; ERG, electroretinogram; FGF-2, fibroblast growth factor; GC1, guanylate cyclase 1; GFP, green fluorescent protein; GNA12, guanine nucleotide α-transducin; HIV, human immunodeficiency virus; HRP, human rhodopsin; IFN, interferon; IL, interleukin; IL-1Ra, interleukin-1 receptor agonist; IOP, intraocular pressure; IRBP, interphotoreceptor retinoid binding protein; IRES, internal ribosome entry site; KO, knockout; MERTK, c-mer proto-oncogene tyrosine kinase; PDE, phosphodiesterase; PEDF, pigment epithelium derived factor; PGK, phosphoglycerate kinase 1; PR2.1., 2.1-kb human red/green opsin promoter construct; pTYF, self-inactivating lentiviral transducing plasmid; RAG-2, recombination activating gene 2; RCS, Royal College of Surgeons; RHO, rhodopsin; RK, rhodopsin kinase; RNAi, RNA interference; RPE, retinal pigment epithelium; RPE65, retinal pigment epithelium-specific protein 65kDa; RPGR, retinitis pigmentosa GTPase regulator; RPGRIP, RP GTPase regulator interacting protein; RSI, retinoschisin; S-Ag, S-antigen; SFFV, spleen focus forming virus; sFLT1, soluble vascular endothelial growth factor receptor; SIN, self-inactivating, replication-deficient lentivirus; SIV, simian immunodeficiency virus; tetON, tetracycline inducible promoter; TIMP3, tissue inhibitor of metalloproteinase 3; TK, thymidine kinase.