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Promising and delivering gene therapies for vision loss

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Abstract

The maturity in our understanding of the genetics and the pathogenesis of disease in degenerative retinal disorders has intersected in past years with a novel treatment paradigm in which a genetic intervention may lead to sustained therapeutic benefit, and in some cases even restoration of vision. Here, we review this prospect of retinal gene therapy, discuss the enabling technologies that have led to first-in-human demonstrations of efficacy and safety, and the road that led to this exciting point in time.

Keywords

Retina; gene therapy; AAV; lentiviral vector; LCA; clinical trial

1. Clinical Success, in triplicate

1.1. Clinical success in Leber Congenital Amaurosis due to mutations in RPE65

Three independent clinical trials for retinal pigment epithelium-specific 65 kDa protein (RPE65) deficiency in 2008[1-4] have led to genuine excitement and anticipation from both the scientific and lay communities towards the treatment of recessive monogenetic disorders that cause vision loss. Mutations in RPE65 leads to early onset vision loss within the disease spectrum referred to as Leber Congenital Amaurosis (LCA). LCA-RPE65 patients generally present with significantly decreased vision in the first year of life, nystagmus, and fundus changes consistent atrophy of the pigment epithelium. The *RPE65* gene encodes an isomerase protein that is expressed in the retinal pigment epithelium (RPE) and is an essential player in the recycling pathway of 11-*cis*-retinal in the visual cycle. Briefly, light activation of the visual pigments (opsins) present in the outer segments of photoreceptors occurs after photon capture by the 11-*cis*-retinal chromophore triggering an isomerization event that converts it to all-*trans*-retinal and releases it from the visual pigment (reviewed in [5]). Recovery of the visual cycle after light stimulation is therefore dependent on the

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conversion, in the RPE, of the chromophore from all-*trans*-retinal to 11-*cis*-retinal by the RPE65 protein[5]. The re-converted 11-*cis*-retinal chromophore will now. travel back to the photoreceptors outer segments and re-attached itself to the visual pigments[5].

The first effective intervention using adeno-associated virus (AAV)-based gene therapy in an animal model of retinal dystrophy caused by an RPE defect was done in the Briard dog model which has a naturally occurring mutation in the *RPE65* gene. AAV2/2 mediated gene transfer after subretinal injections shown significant morphological and functional rescue of photoreceptors and therefore recovery of 11-*cis*-retinal recycling by the RPE cells[6-9]. These studies showed functional ERG improvement of around 20-30% of wildtype levels and significant improvements in behavioral-based vision tests, especially under photopic conditions[6,8,9]. They were also able to demonstrate stable and long-term restoration of vision up to four years follow-up post-treatment[10,11]. These initial studies in a large animal model of RPE65 deficiency that mimicked the human LCA condition so well provided great encouragement and an ideal candidate to move a gene therapy platform for inherited retinal dystrophies towards the clinic.

The reports of the early stage clinical trials for RPE65 deficiency were encouraging and attested for both safety and efficacy of the transgene and the selected AAV2/2 vector delivery agent (reviewed in [12]). Some of the differences between the three trials include vector sequence and design, dose (ranging from 1.5×10^{10} to 1.5×10^{11} viral particles) and injected volume (ranging from 0.15 to 1 ml). Vector production methods also varied between the trials but it is unclear if and how this affects the outcome (see [2] for summary table of differences between trials).

With over a hundred disease-causing mutations identified so far in RPE65 (source: www.retina-international.org), it was unsurprising that all the initial and subsequent patients selected for the trials presented a diverse group of mutations[2,13]. Identical homozygous mutations was only seen in two patients of the Maguire *et a*[3] trial (E102K) and between one patient in the Hauswirth *et al*[2] and Bainbridge *et al*[1] trials (Y368H). Even after these initial studies were expanded[14,15], the diversity of both homozygote and compound mutations in the recruited patients remained high. This has made it difficult so far to correlate specific mutations with visual improvement outcomes, therefore studying the effects each mutation has on RPE65 function needs to be an ongoing effort and run in parallel to clinical trial data.

Arguably the most significant difference between these trials that may have influenced outcome was the choice and design of promoter driving RPE65 expression. Although all three trials used the recombinant AAV2/2 vector (rAAV), Bainbridge *et al*[1] used a human RPE65 promoter while both Maguire *et al*[3] and Hauswirth *et al*[2] used a modified version of the ubiquitous chicken β actin promoter referred to as CAG promoter[16]. Other vector sequence and design differences include the addition of an optimized Kozak sequence in Maguire *et al*[3] trial. Although the human RPE65 promoter has a weaker expression pattern when compared to the CAG promoter, it was shown to drive enough transgene expression to rescue the phenotype of both younger and older treated briard dogs[8,9,17]. These preclinical studies showed that the human RPE65 promoter was capable of driving RPE-specific expression of the transgene, which was opted to be preferred in terms of safety in this study.

In contrast, a ubiquitous promoter like CAG with a non-specific cell expression profile could generate concerns about RPE65 expression in cell types other than RPE and what effect this would have in the recovery of the visual cycle. However, a ubiquitous promoter has its advantages, offering a more robust and stronger expression pattern.

The promoter choice in the RPE65 clinical trials could offer an explanation for the differences seen between the reported outcomes, and indeed, visual improvements were more robust from the two trials that used the CAG promoter[2,3,14,15]. These included improved visual acuity and pupil response, increased sensitivity and in visual field size and a fixation shift in the extrafoveal treatment in one patient[2,3,15]. Long-term follow up of these studies have demonstrated that stability, safety and efficiency of treatment can persist up to at least 3 years post-treatment[13,15]. However it remains debated whether these studies have been able to show an age-dependent effect of the treatment since the two studies with larger patient cohorts including younger aged patients have reached contradictory conclusions. In the first study Maguire *et al*[14] concludes that treatment at a younger age does have an overall effect on improved visual function although a later study conducted by Jacobson *et al*[15] shows no correlation between age and treatment effect. The younger patients in the first study[14] do indeed show a more consistent improvement in visual sensitivity when compared to the older group of patients where the results were more variable but this could easily be explained by the heterogeneity of disease severity caused by RPE65 deficiency, generating a complex and individualist relationship between disease progression and age. Indeed, a few of the older patients show a similar increase in sensitivity when compared to the younger ones and the visual acuity measurements do not seem to show an age-related correlation. Our conclusion from these data at this early stage of the field is that too many variables (mutation-dependent or idiosyncratic progression of disease, vector, injection parameters, and endpoint measures) between these studies and subjects are at play. That being said, our understanding of disease pathology and the data from these studies indicate a greater benefit from intervention at an earlier stage of the disease process, which is age-related.

Next, the hypothesis was challenged whether gene augmentation therapy in this form of LCA would stem degenerative processes in the outer retina, and ultimately determine whether the benefit observed in these pivotal trials would be long-lived[18]. In this study Cideciyan and colleagues extensively and thoroughly analyzed the natural history of the disease using the patients enrolled in one of the initial trials. They concluded that despite the treatment, disease progression and photoreceptor degeneration remained unchanged and followed the expected natural history (further reviewed in [19]). Surprisingly they also show that the standard binary hallmark of inherited retinal degenerations namely combined dysfunction and degeneration of photoreceptor cells - is different between humans and the main animal model used for the pre-clinical studies of LCA, the Briard dog model. While in humans, dysfunction and photoreceptor degeneration are timely coupled, in dogs impaired visual function occurs well before any degeneration is seen. The authors conclude that the accumulation of certain changes by non-functional RPE65 contributes to the generation of a threshold or window where treatment needs to fall within to have significant impact on photoreceptors degeneration and visual improvement. This indeed aligns well with another study from Cideciyan *et al* where they show that despite significant increase in visual

sensitivity after *RPE65* gene therapy in humans, the kinetics of rod photoreceptors recovery is still impaired and suboptimal[20]. While these studies are thorough in their analysis, the bold and disappointing conclusions have been critiqued and challenged[19,21]. Indeed, the small group sizes, the multitude of variables delineated above, and the very early assessment of long-term benefit within a slow degenerative process make any definitive conclusion difficult. For these reasons the Cideciyan study[18] had to use a novel methodological approach, measuring the thickness of photoreceptors outer nuclear layer (ONL) based on normalized OCT data and age correction between species and degenerative states, to generate a predictive slope of the natural history of RPE65-LCA. However, this approach may have its limitations in accurately modeling long-term progression and treatment effects since only one disease parameter, ONL thickness, was taken into consideration. Patients with RPE65-LCA usually present a highly variable disease progression rate, demonstrated by the weak correlation between age and ONL thickness seen in this study, which only showed a more consistent and stepper correlation when adjusted for age of onset[18]. More importantly, their analyses did not account for the diversity in *RPE65* genetic lesions present in this patient cohort that could have helped to better understand the effect of different mutations on disease progression.

Nonetheless, these studies do emphasize the point that gene augmentation therapy has an inherently delineated therapeutic window between the earliest time intervention can be considered and the point when degenerative processes cannot be reverted and eventually therapeutic target cells are terminally atrophied.

1.2. Further delivering on the promise - the Choroideremia clinical trial

Earlier this year we saw the publication of the results of another phase I/II clinical trial for a different type of inherited retinal degeneration[22]. This trial focused on patients with Choroideremia, an X-linked recessive disorder of the retina and choroid due to the loss of function of the Rab escort protein-1 (REP1). MacLaren and colleagues used an AAV2 expression cassette with the same ubiquitous CAG promoter used in some of the previous RPE65 trials[3,15] but interestingly, added a Woodchuck hepatitis virus post-translational regulatory element (WPRE) downstream of the REP1 encoding gene (*CHM*) cDNA. This regulatory element acts as an enhancer of AAV-mediated transgene expression[23] and although long established in preclinical research and in a few other clinical gene therapy studies, had not been used previously in the clinic for retinal gene therapy approaches.

This study enrolled six patients with null mutations in the *CHM* gene that received 1×10^{10} genome particles of AAV2.REP1 vector in a volume of 0.1ml (except for patient six which received 6×10^{9} particles in 0.06ml) that was injected subretinally and layered under the fovea as a two-step procedure. At the six months follow-up an increase in the point of maximal sensitivity in all treated eyes was observed as well as imovement of mean retinal sensitivity in five out of six treated eyes. Not surprisingly, the two patients with the highest increase in mean sensitivity, although had the smallest area of treatment, received the highest dose of vector per mm^2 of retina. Maximal and mean sensitivities measurements were taken under mesopic light conditions and are therefore more indicative of rod photoreceptor function but they also report an increase in visual acuity in two of the treated

eyes which had the lowest visual acuity baselines. The remaining four patients where no visual acuity improvement was reported also showed no detrimental effects after the treatment which coupled with their near normal visual acuity at baseline highlights the fact that the procedure-related retinal detachment in the fairly healthy macular and foveal area is not a concern in these patients as seen in other gene therapy trials[15]. This is also corroborated by outer retina thickness measurements that remained similar before and at six months post-treatment. One of the most interesting results however was the shift in retinal fixation points in two patients towards the treated area, and in one case, completely bypassing a close by area of residual retina that had not been exposed to the vector. This effect was also seen in one patient treated in one of the RPE65 trials[20] and provides hope for improvements in visual acuity and sensitivity even after the fovea and/or macular regions have undergone extensive and irreversible degeneration.

This is the first report of a gene therapy approach targeted towards gene augmentation in photoreceptor cells. It also shows that vector administration in patients with near normal visual acuity and retinal thickness is safe and does not cause any detrimental effects after the detachment induced by the procedure. Due to the slow degeneration rate of Choroideremia, the normal visual acuity of four out of the six patients and the treatment occurring before a clinically significant retinal thinning, it remains to be seen whether in this case gene therapy is capable of slowing down the progression of photoreceptor loss.

1.3. Other ongoing translational programs

1.3.1. AMD Clinical Trials—Age-Related Macular Degeneration (AMD) is the leading cause of visual loss in adults in Western societies[24]. Its etiology is complex and only in part genetic. Its pathogenesis is fascinating and only partially understood. Early stage AMD is characterized by subretinal deposits called drusen and retinal pigment epithelium (RPE) irregularities in the macula, and often called dry AMD. This pathology leads most often to no or limited vision loss in the affected areas of the retina. Later stages of this disease however are clinically much more significant, and lead to presentations of either large macular areas of degeneration, often referred to as Geographic Atrophy, or a disease characterized by choroidal neovascularization (CNV) which results in exudative or the wet form of AMD. Only for wet AMD a treatment is currently available which requires repeat intravitreal injections of agonist of Vascular Endothelial Growth Factor (VEGF)[25]. In order to overcome the need for repeat injections, and improve on the peak and trough pharmacology of bolus injections, several groups have considered encoding a VEGF antagonist in a gene therapy vector. Two groups, Genzyme-Sanofi and Avalanche (partnered with Lions Eye Institute in Australia), are progressing this approach in the clinic. Both groups are using a very similarly designed vector based on AAV2 encoding soluble forms of secreted ligands of VEGF derived from Flt-1. The Avalanche-Lions Institute trial is using the sFlt1 gene, an alternatively spliced form of the Flt1 protein containing only the extracellular domains[26], while the Genzyme-Sanofi trial has selected the *sFLT01* gene which encodes a hybrid molecule made of the second immunoglobulin (IgG)-like domain of Flt-1 fused to a human IgG1 Fc through a polyglycine linker[27]. The other major distinction between the two efforts is the route of administration with the Genzyme group proposing

intravitreal vector delivery, and Avalanche subretinal. Both studies are currently ongoing and are expected to present results in the near future.

Currently the only clinical trials for visual disorders using lentiviral vectors are being run by Oxford BioMedica UK Ltd (Oxford, UK) and one of them is to treat neovascular AMD by delivering two angiostatic proteins, endostatin and angiostatin, to the eye. They are using an equine infectious anemia virus (EIAV)-based lentiviral gene therapy vector expressing both proteins called RetinoStat©. Long-term safety studies in non-human primates and rabbits have shown that RetinoStat is well tolerated after subretinal injections, generating only a mild and transient ocular inflammatory response[28]. They also show that biodistribution is restricted to ocular structures and stable expression of both endostatin and angiostatin was observed up to six months in rabbit vitreous samples[28]. The phase I clinical trial of RetinoStat will initially test safety and bioactivity of the vector in patients with advance neovascular AMD by assessing baseline changes in size of choroidal neovascular lesions, subretinal and intraretinal fluid and best corrective visual acuity. This trial will not only provide valuable information regarding the effects of endostatin and angiostatin on AMD patients but also test the efficacy and safety of using lentiviral vectors to delivery gene augmentation for visual disorders.

1.3.2 Clinical trials for other inherited retinal degenerations—Besides RetinoStat, Oxford BioMedica UK Ltd also has two more products which they are currently recruiting for clinical trials, StarGen™ and UshStat©, to treat Stargardt macular degeneration and retinitis pigmentosa associated with Usher syndrome type 1B (USH1B), respectively. In line with the AMD trial, StarGen and UshStat are also nonreplicating, nonhuman recombinant lentiviral vectors based on EIAV. The choice of a lentiviral-based vector for these two trials is explained by the size of the transgene to be supplemented. In both disorders, the disease-causing mutations are found in genes which exceed the maximum genetic load AAV-based vectors are capable of packaging, therefore creating the need for alternative gene delivery platform (see also section 3.2 below on large genomes).

StarGen expresses the large human photoreceptor-specific adenosine triphosphate (ATP)-binding cassette transporter (*ABCA4*) gene under the regulation of a constitutively active CMV promoter and will be delivered subretinally to target rod and cone photoreceptors. Preclinical safety and biodistribution of StarGen was evaluated by Binley and Colleagues[29] in rabbits and macaques and shown to have expression restricted to ocular tissues and no antibodies to StarGen vector components was detected in macaque serum. However, they report that in three out of six rabbit tissue samples, vector DNA was detected in the optic nerve. This could indicate a possible somewhat inefficient targeting of retinal ganglion cells suggesting a non-photoreceptor specific expression of their vector. Factors like the ubiquitous CMV promoter, vector tropism in rabbit ocular tissue and the nature of vesicular stomatitis virus envelope used to pseudotype the vector particles could all play a part in these results and it is still unclear whether this could negatively impact on the clinical trial outcome.

UshStat will be used to treat progressive retinitis pigmentosa in USH1B patients that have mutations in the gene encoding a myosin motor protein, myosin VIIA (*MYO7A*). Preclinical

proof of concept studies in the *shaker1* mouse model of USH1B, which carries a mutated copy of the *Myo7a* gene, and in non-human primates has shown that the UshStat vector, carrying a transgene cassette composed of the human *MYO7A* gene under the control of a CMV promoter, is capable of successfully transducing both mouse and monkey retinas after subretinal injections[30]. Furthermore, UshStat was able to prevent light-induced retinal degeneration. Similar to what was reported with RetinoStat, a transient ocular inflammatory response was seen in monkeys but no humoral antibody response to either the *MYO7A* transgene or vector components was found[30]. The results from the clinical trials for both UshStat and StarGen will hopefully help assess the long-term safety, tolerability and biological activity of these vectors alongside treatment efficacy and will be extremely useful in evaluated the suitability of using a lentiviral-based platform for retinal therapy of large genes.

In February 2014 yet another clinical trial was initiated and is currently recruiting patients to test the efficacy of AAV2/2-based gene therapy for a mitochondrial disease, Leber hereditary optic neuropathy (LHON), which due to mutations in the *ND4* gene causes degeneration of the retinal ganglion cells (RGC) and atrophy of the optic nerve and therefore vision loss (reviewed in [31]). Led by the company Gensight, they will be testing their main product, GS010, in a Phase 1 and 2 dose-escalation trials to evaluate safety and tolerability profile of their product in LHON patients. The GS010 is an AAV2/2 based vector encoding the mitochondrial NADH dehydrogenase 4 (*ND4*) gene and will be delivered intravitreally to target the RGCs. Mitochondrial targeting will be achieved by the fusion of the optimized human wild-type *ND4* Open Reading Frame (ORF) to a *COX10* fragment which encodes a N-terminal mitochondrial targeting sequence (MTS) plus additional residues to ensure MTS cleavage afterwards[32]. Adding to the Genzyme/Sanofi AMD trial, this will be another effort towards intravitreally-delivered AAV2/2 to provide gene augmentation under two different contexts and the results will be eagerly awaited.

2. Preclinical promise

2.1 Proof-of-concept in individual gene targeted therapies

These first clinical trials were preceded by just over a decade of intensive research therapeutic gene transfer to the retina which saw the efficiency of treatment in animal models grow exponentially from the first study done in the fast retinal degeneration Pde6b-deficient rd1 mouse model in 1997, showing only a minor histological rescue of a few rows of photoreceptors[33], to some recent studies where near wild type level of functional rescue has been reported[34-37]. The eye has several features that make it an ideal candidate for gene therapy. It is a small and immune-privileged organ where the blood-retina barrier separates the ocular space from the blood supply reducing therefore immune response to the vectors. The compartmentalized and structured anatomy of the retina also allows for controlled and localized delivery of relatively small amounts of the therapeutic vector through a moderately easy surgical procedures and treatment outcomes can be easily accessed by non-invasive methods such as electroretinography (ERG) and optical coherence tomography (OCT). The non-dividing character of both RPE and photoreceptors coupled with a plethora of inherited

retinal diseases encouraged the scientific community to take active steps towards developing a gene therapy platform to treat vision loss.

The very first efforts to target the retina for gene delivery were done using replication-deficient adenovirus and showed that the RPE layer, but only a few individual photoreceptors, was quite permissive to viral transduction[38]. Further studies tried to improve retinal transduction by adenovirus but only temporary expression of the transgene was observed[39-41]. A few years later came the first report of efficient retinal cell transduction by AAV-based vectors, showing high RPE expression of the reporter gene by 28 days post-injection[42]. Shortly after more permanent and long-term retinal expression was observed when Miyoshi and Colleagues[43] used an HIV-based lentiviral vector containing a green fluorescent protein (GFP) under the control of either the ubiquitous CMV or photoreceptor-specific Rhodopsin promoter to successfully transduce RPE and photoreceptor cells. However due to toxicity, immunogenicity, and other safety concerns of adenoviral or lentiviral vectors, AAV-based vectors quickly became a clear leader in the race for retinal gene therapy (reviewed in [44]).

The next few years saw a surge in retinal gene transfer studies where several rodent and large animal models were shown to be amenable to histological and functional rescue of RPE and photoreceptor layers after AAV transgene delivery (reviewed in [45-50]). These studies were able to show a widely heterogeneous behavior of AAV vectors ranging from differing retinal tropism amongst serotypes, route of vector delivery, timing of injection and transduction efficiencies[51]. More recently technological advances have also help improve gene delivery to the retina; continual discovery of new serotypes and controlled mutations of capsid residues have dramatically increased retinal transduction patterns[52,53] and a better understanding of the kinetics of subretinal AAV delivery have also aided in better study design and therefore more efficient gene transfer to the retina[54-57]. However translating the results from preclinical animal model studies to the clinic has proven difficult and slow due to biological limitations imposed by the current technologies or disease process, the lengthy, expensive, and complex path of bringing a complex biologic to a clinical trial, and the fact that many of these individual inherited retinal disorders are extremely rare. Table 1 documents those efforts that successfully navigate this translational path, and shows a summary of current ongoing trials for retinal disorders. With hundreds of disease genes within the inherited retinal disease spectrum, dozens of which preclinical proof-of-concept rescue through gene augmentation therapy has been established, and the pivotal first steps in clinical demonstrations of efficacy and safety shown, the question remains on how the field can bring therapies to the clinic for many more of these often indications that individually often affect only relatively small populations.

2.2 Gene-independent treatment paradigms

The primary concept of optogenetics relies on the possibility of delivering light-sensitive molecules to a retina where degeneration is so advanced that all other therapies like gene replacement and stem cell therapy are past their optimal window for treatment and therefore might prove inefficient. A non-endogenous light sensitive molecule could then be targeted to what surviving cells are remaining, and therefore not susceptible to the degeneration, but are

naturally incapable of responding to light stimulus. For this approach to be successful there is a need for a good light-sensing molecule that ideally does not require the presence of a detachable chromophore-activating G-protein cascade, as is the case with some opsin pigments, since the cells usually targeted in optogenetics approaches are incapable of metabolising and recycling chromophores. The answer to this was found in bacteria where rhodopsin-like molecules, through reversible photoisomerization of their chromophore group can directly generate ion moment through the membrane creating an electrical signal in response to light stimulation[58].

The first study to use an optogenetic approach to restore light-evoked response to the degenerate retina was published in 2006 by Bi and Colleagues who showed that AAV2/2-based delivery of a microbial-type rhodopsin called channelrhodopsin-2 (ChR2) to the neurons of the mouse inner nuclear retinal layer was capable of successfully generating light-evoked voltage responses of ChR2-expressing retinal neurons, both at the retinal and cortical levels, to retinal light stimulation in the rd1 mouse[59]. Since the publication of this first study, many investigators have been compelled by the power of bringing the technology of gene therapy together with that of optogenetics to restore light sensitivity and visual function to the retina in the blind. Others have extensively reviewed this approach, and highlighted the refinements and advances that will be necessary to safely achieve this ambitious approach in restoring clinically meaningful visual perception[60,61].

Other gene-independent treatments that have used to successfully prevent photoreceptors cell loss include retinal delivery of neurotrophic factors during retinal degeneration. The rationales behind these studies aim at prolonging photoreceptors survival and therefore generating a better window for vector gene-delivery treatments but also include the premise that survival of rod photoreceptors will also prolong cone survival from secondary death mechanisms. The best results have been achieved using AAV-delivered ciliary neurotrophic factor (CNTF), basic fibroblast growth factor (FGF) and glial cell– derived neurotrophic factor (GDNF)[62-67]. However the use of CNTF to prevent retinal degeneration has proven controversial since some studies have shown an adverse and/or dose-dependent effect[65-67] and clinical trials using intraocular implant-delivered CNTF in RP patients has reported no therapeutic improvement[68].

3. Technological innovation for current hurdles

3.1. Improving delivery and surgical access

In most active clinical studies the therapeutic vector is delivered by a subretinal injection (SR). This approach relies on the infusion of vector in a virtual space between the outer nuclear layer and RPE through the generation of an iatrogenic retinal detachment. This unorthodox surgical intervention however has been key to the success of retinal gene therapy efforts, particularly those targeting ONL and RPE; vector is positioned in close proximity of relevant therapeutic target cells, remains in a contained space not subject to significant dilution or biodistribution, and in preclinical models has been shown to be far superior in terms of efficiency as compared to any other route of administration.

Surgically, first the vitreous is removed via a three-port *pars plana* vitrectomy. Next, a subretinal cannula is inserted below the retina where the therapeutic agent is injected into the subretinal space (videos in [13]). This translates to a focal area of treatment (referred to as the bleb) and allows positioning of the vector e.g. centrally or peripherally. Remarkably, from experience in small and large animal models, the bleb resolves relatively fast and leads to a quiet retina in less than a few days in most cases. Indeed the procedure was found to be overall safe in humans too in the RPE65 clinical trials where in most patients no surgical adverse effects were reported[1-3,14,20]. The adverse effects that were observed occurred in the immediate postoperative days and included retinal detachments, foveal thinning, macular holes, choroidal effusions and ocular hypo- and hypertension[3,15]. Of these, only the macular hole (one patient)[3] and one case of persistent choroidal effusions[15] were not resolved after 30-60 days post-surgery. While overall subretinal injection appears to be well tolerated, they are complex with inherent variables from patient to patient, lengthy, and require unique and expert surgical skill. Although the majority of the clinical trials data show no detrimental effect of the surgery on the retina, one study reported that two out of five patients where the injected area included the fovea showed foveal thinning at early postoperative time points[15]. This was concluded to be due to damage from the surgical retinal detachment as opposed to foveal thinning at later time points as a consequence of natural progression of disease[15].

Although most studies do not report retinal thinning and major complication due to the surgical procedure[1,22,69], this effect, or lack thereof, could be disease-related and therefore dependent on how preserved and stable are the interactions between RPE and photoreceptor layers in each case. Despite the promising evidence from the Choroideremia trial where patients with near normal visual acuity underwent the surgical procedure without significant loss of acuity[22], it still remains unclear if this is related to the specific genetic defect of these patients or just related to retinal and foveal thickness at the time of surgery. Therefore, this might become an increasingly vexing question with the possibility of pre-treatment of genetic retinal defects; how willingly would asymptomatic patients accept a treatment that results in retinal damage and potential loss of vision acuity to save them from more severe damage in the future? This has generated efforts towards the development and optimization of a less invasive alternative to deliver gene therapy vectors to the eye and the alternative delivery method, which is less invasive and would not require a retinal detachment, would be through intravitreal injections (IVT). With this procedure the gene delivery agent can be injected into the vitreous of the patients and diffused into the retinal layers from within the vitreous cavity bypassing several of the risks involved with a subretinal injection.

Regardless of the individual risks of each type of surgery, the most important difference between the two methods of gene delivery probably lies in their vector transduction and diffusion pattern. Through a subretinal injection each individual cell transduced in the subretinal space will be exposed to varying levels of the therapeutic agent; cells in the center of the bleb will be transduced by a much higher number of particles than the ones in the periphery. This would be advantageous, for example, when targeting diseases that affect the macula, where a focal high dose delivery would increase the chances of rescue. However for disorders where widespread retinal degeneration is seen, like in RP patients for example, an intravitreal approach could offer a more appropriate delivery of the vector, targeting a larger

population of cells with more uniform transduction levels. This method would ideally offer a much wider, pan-retinal homogeneous transduction area. Whether better vision restoration would be achieved via a concentrated focal versus a diluted widespread delivery remains a very interesting discussion point that still needs to be evaluated on several different parameters to provide an answer.

However, transduction efficacy of rAAV vectors delivered from the vitreous have had limited success in the past, with AAV2/2 achieving the highest number of transduced retinal cells in mouse, dogs and non-human primates compared to several other serotypes[70,71]. However, the impediment for successful transduction of the retinal cell layers via intravitreal injections are thought to be the vitreous jelly, the physical barrier created by the inner limiting membrane (ILM) and the complex tangle of different cells and processes that form the vitreous and inner retina[70]. Nevertheless recent efforts to improve cell transduced after intravitreal injection have been met with encouraging success. Recently a study led by Drs Schaffer and Flannery at the University of California, Berkeley, used an *in vivo*-directed evolution approach to select, from a genetically engineered capsid AAV library, variants capsids that were able to transduced different retinal cell types via an intravitreal injection[72,73]. After a few rounds of *in vivo* selection, they were able to select AAV capsids that showed a high transduction efficiency of different retinal layers using either a ubiquitous or rod-specific promoter[72,73]. The best AAV variants from these studies, 7m8 and ShH10, have already been used in subsequent studies demonstrating effective gene delivery and functional rescue of the retinoschisin protein-deficient *Rs1h*$^{-/-}$ mouse model of X-linked retinoschisis[74]. Other studies using intravitreal delivery to target retinal cells have used targeted mutations of AAV capsid residues showing that a five mutation AAV2 capsid can efficiently target photoreceptors in the mouse retina[75]. Data from these studies demonstrates increased efficiencies of retinal targeting from this clinically more attractive route of administration in mice, however experience in larger animal models have been less encouraging, likely due to the barriers to transduction that are more pronounced in larger eyes.

3.2. Size matters

As demonstrated by the results from the clinical trials and years of pre-clinical research, safety and efficacy has made AAV the vector of choice for gene augmentation in retina. However, a major shortcoming is its small genome size, restricting the amount of the packaged transgene to around 4.7 kilobases[76]. This small genome capacity thus becomes a limiting factor for devising current gene replacement therapy for many important causes of disease, such as mutations in the CEP290, MYO7A or RP1 genes, which have cDNAs significantly larger than the carrying capacity of AAV. Even in the cases of smaller genes, the use of specific promoters and other minimal regulatory elements is often necessary, resulting in compromises on transgene expression levels and dynamics of regulation. Even though larger packaging viruses and naked DNA delivery systems like nanoparticles could provide an alternative approach and circumvent this problem, no other vector system has been defined that rivals the efficiency of gene transfer and the safety profile of AAV.

Therefore the pursuit to increase the carrying capacity of the AAV has generated considerable interest from the scientific community recently. Based on the capacity of AAV circular genomes to concatamerize via intermolecular recombination[77], Yan and colleagues were the first to demonstrate that two independent *Trans*-splicing rAAV vectors encoding non-overlapping segments of the large erythropoietin (Epo) gene and intron donor and acceptor sites were able to express protein at therapeutic levels[78]. This dual vector approach was later expanded to include other strategies for gene reconstitution like sequence overlap[79], a triple *Trans*-splicing[80] and a hybrid of *Trans*-splicing and sequence overlap with the hybrid strategy showing the highest efficiency[81]. Also subsequent studies have shown protein expression and functional *in vivo* rescue after reconstitution of other large genes like dystrophin[82,83] and dysferlin[84,85].

There have also been some tentative efforts to use these dual AAV strategies to develop gene replacement therapy for some cases of retinal degeneration, mainly those caused by mutations in *ABCA4*, *CEP290* and *MYO7A*[86-88]. Recessive mutations in MYO7A are responsible for around half of all Usher syndrome type 1 cases but with a cDNA of almost 7kb it has remained an elusive candidate for gene replacement therapy. Initial results for *MYO7A* gene delivery in the retina showed that AAV2 or AAV5 encoding the full-length gene was more efficient both *in vitro* and *in vivo* when compared to a dual vector overlap approach[86]. Although certain serotypes were reported to be able to package genomes up to 8 kb[89], follow-up studies revealed that the resulting transgene activity was actually due to separately packaged heterogeneous population of fragmented AAV genomes reconstituting the full-length transgene[90-92]. Although Lopes and colleagues do suggest that MYO7A expression is probably due to successful recombination of overlapping fragments, they are unable to explain the low reconstitution rate of the dual overlap AAV strategy[86]. A recent paper by Trapani *et al* has shed some light into this issue by showing that mouse and pig photoreceptors can be efficiently transduced by trans-splicing and hybrid dual AAVs but not by overlapping vectors[93]. This was again confirmed later in another study when MYO7A levels in mouse retinas injected subretinally with either overlapping or hybrid dual AAVs were only detectable, by western blotting, with the hybrid approach[87].

Although *in vivo* retinal levels of protein after subretinal administration of dual trans-splice or hybrid AAVs has been shown to be around 20% of endogenous levels[93], dual AAV vector approaches have not gained major popularity since circumventing the AAV carrying capacity problem in this manner severely impacts transgene expression efficiencies and it is still unclear why fragment reconstitution levels are so inefficient.

3.3. To AAV, or not to AAV

Regardless of the extremely successful recent history of AAV-mediated gene therapy for several ocular disorders, the current technologies still limit translation for many indications to which we have not yet found solutions for. One of the main restrictions of AAV-based vectors is the limited size of the therapeutic cargo it can package, and is often the primary reasons to consider alternative delivery strategies. AAV is generally seen as of low immunogenic potential, certainly compared to adenoviral vectors, however the preclinical lead up of Genzyme's wet AMD AAV2 clinical study encountered moderate, self-limiting

yet long lasting inflammation in the vitreous[94]. This may or may not be a function of the fact that AAV is endemic in primates, and an AAV vector dosing would elicit a memory response and/or encounter pre-existing immunity. The latter was studied in a sampling of vitreous fluid in which low level neutralizing anti-AAV antibodies were detected that could interfere with retinal AAV gene transfer[95]. Despite the overwhelming number of AAV-based studies for gene therapy in the eye, lentiviral vectors offer an attractive alternative due to their ability to transduce diving and non-diving cells stably and have a much larger genome packaging capacity compared to AAV vectors. The most widely used vectors currently are derived from the human/simian immunodeficiency virus (HIV/SIV) or the equine infectious anemia virus (EIAV). Besides their ability to carry larger genome they can also undergo a pseudotype process where envelope proteins can be exchanged with that of other viruses therefore modifying its tropism (reviewed in [96]).

Initial vectors derived from retroviruses were only able to transduce dividing cells and were therefore very inefficient on post-mitotic adult photoreceptors and RPE cells, restricting its applications to *ex vivo* gene transfer[97,98]. The later development of a modified replication-deficient HIV-derived retroviral vector allowed the transduction of non-dividing cells[99] and was subsequently shown to successfully target photoreceptors and RPE cells, cornal endothelium and trabecular meshwork[43,100,101]. However the percentage of transduced mouse photoreceptors after lentiviral gene delivery still remains higher in newborn retinas compared to adult mice[102]. One explanation was offered by Gruter *et al*[103] who showed improved photoreceptor transduction after treatment with chondroitinase or neurominidase, therefore suggesting that a physical barrier could be preventing virus diffusion through the retina. Despite the drawbacks of lentiviral-based vectors for inherited retinal disorders, there are currently three ongoing clinical trials using EIAV-based vectors, two to deliver large genes to photoreceptors subretinally and the other encoding the genes for two angiostatic proteins for intravitreal delivery (see sections 1.3.1 and 1.3.2 above). The outcome of these trials will be closely followed by the scientific community since they might provide alternative platforms for gene therapy for vision loss.

A promising new approach that appears to be able to overcome some of these issues is the use of nanoparticles to deliver therapeutic agents to target tissues. These non-viral delivery systems may offer a less invasive, low immunogenic and inflammatory response and can package plasmids up to 20kb, therefore holding great potential as a future therapeutic option. They mainly involve cationic lipids or polymers which then form lipoplexes or polyplexes around a DNA molecule and have been shown to successfully deliver DNA particles to different ocular tissues[104-108]. However as a tool for ocular gene therapy these polymeric vectors still need to overcome several issues prior to translational applications. Some of these include permeation barriers like the vitreous and the blood-retinal barriers, low efficiency, short half-life and transient gene expression and non-targeted delivery (reviewed in[109,110]). Non-viral vectors do hold future potential for becoming a viable option for ocular gene therapy but further studies into the issues mentioned above are required before serious consideration in a clinical setting.

4. Looking ahead

In vivo gene therapy has been pursued for several decades, and now recently has reached a point where patient benefit has been demonstrated in a discreet number of retinal clinical trials for two blinding disorders. The validation of this potentially powerful approach has generated excitement to develop more gene treatments for inherited forms of blindness. In fact, using the same technology and lessons learned from the first pivotal studies, it has been proposed to build a platform of which all components of the therapeutic vector are validated, and only the therapeutic gene cargo is exchanged depending on the indications[111]. It will indeed be necessary to streamline the development and regulatory hurdles for these types of therapies if ever the field wants to target therapeutics to the multitude retinal dystrophies. This is particularly true for those very rare indications for which gene therapy has the potential to be beneficial, however due to the limited prevalence, the costly pursuit of building and developing a treatment is economically difficult to find traction for. To build a platform as described above, progress has to be made in several aspects of this intricate field of converging expertise, some of which are addressed above. In addition to working out methods and technologies to improve the safety and efficacy of this still experimental approach, it is becoming increasingly important to find better ways to capture accurately and with great sensitivity the therapeutic benefit that a gene therapy may bring to the patient. This will require extensive natural history studies for each indication to map, and ultimately predict, disease progression, validation of clinical endpoints (including imaging modalities and biomarkers), and with those data at hand, an effort to convince regulatory agencies that these can measure a meaningful benefit to the patients.

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References

1. Bainbridge JW, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med. 2008; 358:2231–2239.10.1056/NEJMoa0802268 [PubMed: 18441371]
2. Hauswirth WW, 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; 19:979–990.10.1089/hum.2008.107 [PubMed: 18774912]
3. Maguire AM, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. The New England journal of medicine. 2008; 358:2240–2248.10.1056/NEJMoa0802315 [PubMed: 18441370]
4. Cideciyan AV, et al. Human gene therapy for RPE65 isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:15112–15117.10.1073/pnas.0807027105 [PubMed: 18809924]
5. Ebrey T, Koutalos Y. Vertebrate photoreceptors. Progress in retinal and eye research. 2001; 20:49–94. [PubMed: 11070368]
6. Acland GM, et al. Gene therapy restores vision in a canine model of childhood blindness. Nat Genet. 2001; 28:92–95.10.1038/88327 [PubMed: 11326284]

7. Narfstrom K, et al. Functional and structural recovery of the retina after gene therapy in the RPE65 null mutation dog. Investigative ophthalmology & visual science. 2003; 44:1663–1672. [PubMed: 12657607]
8. Le Meur G, et al. Restoration of vision in RPE65-deficient Briard dogs using an AAV serotype 4 vector that specifically targets the retinal pigmented epithelium. Gene therapy. 2007; 14:292–303.10.1038/sj.gt.3302861 [PubMed: 17024105]
9. Rolling F, et al. Gene therapeutic prospects in early onset of severe retinal dystrophy: restoration of vision in RPE65 Briard dogs using an AAV serotype 4 vector that specifically targets the retinal pigmented epithelium. Bulletin et memoires de l'Academie royale de medecine de Belgique. 2006; 161:497–508. discussion 508-499.
10. Acland GM, et al. Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. Molecular therapy: the journal of the American Society of Gene Therapy. 2005; 12:1072–1082.10.1016/j.ymthe.2005.08.008 [PubMed: 16226919]
11. Narfstrom K, et al. Morphological aspects related to long-term functional improvement of the retina in the 4 years following rAAV-mediated gene transfer in the RPE65 null mutation dog. Advances in experimental medicine and biology. 2008; 613:139–146. [PubMed: 18188938]
12. MacLaren RE. An analysis of retinal gene therapy clinical trials. Curr Opin Mol Ther. 2009; 11:540–546. [PubMed: 19806502]
13. Testa F, et al. Three-year follow-up after unilateral subretinal delivery of adeno-associated virus in patients with Leber congenital Amaurosis type 2. Ophthalmology. 2013; 120:1283–1291.10.1016/j.ophtha.2012.11.048 [PubMed: 23474247]
14. Maguire AM, et al. Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. Lancet. 2009; 374:1597–1605.10.1016/S0140-6736(09)61836-5 [PubMed: 19854499]
15. Jacobson SG, 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. Archives of ophthalmology. 2012; 130:9–24.10.1001/archophthalmol.2011.298 [PubMed: 21911650]
16. Miyazaki J, et al. Expression vector system based on the chicken beta-actin promoter directs efficient production of interleukin-5. Gene. 1989; 79:269–277. [PubMed: 2551778]
17. Annear MJ, et al. Successful gene therapy in older Rpe65-deficient dogs following subretinal injection of an adeno-associated vector expressing RPE65. Hum Gene Ther. 2013; 24:883–893.10.1089/hum.2013.146 [PubMed: 24028205]
18. Cideciyan AV, et al. Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110:E517–525.10.1073/pnas.1218933110 [PubMed: 23341635]
19. Cepko CL, Vandenberghe LH. Retinal gene therapy coming of age. Human gene therapy. 2013; 24:242–244.10.1089/hum.2013.050 [PubMed: 23458444]
20. Cideciyan AV, et al. Human RPE65 gene therapy for Leber congenital amaurosis: persistence of early visual improvements and safety at 1 year. Hum Gene Ther. 2009; 20:999–1004.10.1089/hum.2009.086 [PubMed: 19583479]
21. Wojno AP, Pierce EA, Bennett J. Seeing the light. Science translational medicine. 2013; 5:175–178.10.1126/scitranslmed.3005798
22. MacLaren RE, et al. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. Lancet. 2014; 383:1129–1137.10.1016/S0140-6736(13)62117-0 [PubMed: 24439297]
23. Loeb JE, Cordier WS, Harris ME, Weitzman MD, Hope TJ. Enhanced expression of transgenes from adeno-associated virus vectors with the woodchuck hepatitis virus posttranscriptional regulatory element: implications for gene therapy. Human gene therapy. 1999; 10:2295–2305.10.1089/10430349950016942 [PubMed: 10515449]
24. Aleman TS, et al. Retinal laminar architecture in human retinitis pigmentosa caused by Rhodopsin gene mutations. Invest Ophthalmol Vis Sci. 2008; 49:1580–1590.10.1167/iovs.07-1110 [PubMed: 18385078]

25. Holz FG, Schmitz-Valckenberg S, Fleckenstein M. Recent developments in the treatment of age-related macular degeneration. The Journal of clinical investigation. 2014; 124:1430–1438.10.1172/JCI71029 [PubMed: 24691477]
26. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci U S A. 1993; 90:10705–10709. [PubMed: 8248162]
27. Pechan P, et al. Novel anti-VEGF chimeric molecules delivered by AAV vectors for inhibition of retinal neovascularization. Gene Ther. 2009; 16:10–16.10.1038/gt.2008.115 [PubMed: 18633446]
28. Binley K, et al. Safety and biodistribution of an equine infectious anemia virus-based gene therapy, RetinoStat((R)), for age-related macular degeneration. Human gene therapy. 2012; 23:980–991.10.1089/hum.2012.008 [PubMed: 22716662]
29. Binley K, et al. Transduction of photoreceptors with equine infectious anemia virus lentiviral vectors: safety and biodistribution of StarGen for Stargardt disease. Investigative ophthalmology & visual science. 2013; 54:4061–4071.10.1167/iovs.13-11871 [PubMed: 23620430]
30. Zallocchi M, et al. EIAV-Based Retinal Gene Therapy in the shaker1 Mouse Model for Usher Syndrome Type 1B: Development of UshStat. PLoS One. 2014; 9:e94272.10.1371/journal.pone.0094272 [PubMed: 24705452]
31. Cwerman-Thibault H, Augustin S, Ellouze S, Sahel JA, Corral-Debrinski M. Gene therapy for mitochondrial diseases: Leber Hereditary Optic Neuropathy as the first candidate for a clinical trial. Comptes rendus biologies. 2014; 337:193–206.10.1016/j.crvi.2013.11.011 [PubMed: 24702846]
32. Ellouze S, et al. Optimized allotopic expression of the human mitochondrial ND4 prevents blindness in a rat model of mitochondrial dysfunction. American journal of human genetics. 2008; 83:373–387.10.1016/j.ajhg.2008.08.013 [PubMed: 18771762]
33. Jomary C, Vincent KA, Grist J, Neal MJ, Jones SE. Rescue of photoreceptor function by AAV-mediated gene transfer in a mouse model of inherited retinal degeneration. Gene Ther. 1997; 4:683–690.10.1038/sj.gt.3300440 [PubMed: 9282169]
34. Carvalho LS, et al. Long-term and age-dependent restoration of visual function in a mouse model of CNGB3-associated achromatopsia following gene therapy. Hum Mol Genet. 2011; 20:3161–3175.10.1093/hmg/ddr218 [PubMed: 21576125]
35. Mihelec M, et al. Long-term preservation of cones and improvement in visual function following gene therapy in a mouse model of leber congenital amaurosis caused by guanylate cyclase-1 deficiency. Hum Gene Ther. 2011; 22:1179–1190.10.1089/hum.2011.069 [PubMed: 21671801]
36. Tan MH, 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. Human molecular genetics. 2009; 18:2099–2114.10.1093/hmg/ddp133 [PubMed: 19299492]
37. Dai X, et al. AAV-Mediated Lysophosphatidylcholine Acyltransferase 1 (Lpcat1) Gene Replacement Therapy Rescues Retinal Degeneration in rd11 Mice. Invest Ophthalmol Vis Sci. 2014; 55:1724–1734.10.1167/iovs.13-13654 [PubMed: 24557352]
38. Bennett J, Wilson J, Sun D, Forbes B, Maguire A. Adenovirus vector-mediated in vivo gene transfer into adult murine retina. Investigative ophthalmology & visual science. 1994; 35:2535–2542. [PubMed: 8163342]
39. Li T, et al. In vivo transfer of a reporter gene to the retina mediated by an adenoviral vector. Investigative ophthalmology & visual science. 1994; 35:2543–2549. [PubMed: 8163343]
40. Mashhour B, Couton D, Perricaudet M, Briand P. In vivo adenovirus-mediated gene transfer into ocular tissues. Gene therapy. 1994; 1:122–126. [PubMed: 7584067]
41. Bennett J, et al. Photoreceptor cell rescue in retinal degeneration (rd) mice by in vivo gene therapy. Nature medicine. 1996; 2:649–654.
42. Ali RR, et al. Gene transfer into the mouse retina mediated by an adeno-associated viral vector. Human molecular genetics. 1996; 5:591–594. [PubMed: 8733124]
43. Miyoshi H, Takahashi M, Gage FH, Verma IM. Stable and efficient gene transfer into the retina using an HIV-based lentiviral vector. Proc Natl Acad Sci U S A. 1997; 94:10319–10323. [PubMed: 9294208]

44. Bainbridge JW, Tan MH, Ali RR. Gene therapy progress and prospects: the eye. Gene therapy. 2006; 13:1191–1197.10.1038/sj.gt.3302812 [PubMed: 16838031]
45. Buch PK, Bainbridge JW, Ali RR. AAV-mediated gene therapy for retinal disorders: from mouse to man. Gene therapy. 2008; 15:849–857.10.1038/gt.2008.66 [PubMed: 18418417]
46. Dinculescu A, Glushakova L, Min SH, Hauswirth WW. Adeno-associated virus-vectored gene therapy for retinal disease. Human gene therapy. 2005; 16:649–663.10.1089/hum.2005.16.649 [PubMed: 15960597]
47. Smith AJ, Bainbridge JW, Ali RR. Gene supplementation therapy for recessive forms of inherited retinal dystrophies. Gene therapy. 2012; 19:154–161.10.1038/gt.2011.161 [PubMed: 22033465]
48. McClements ME, MacLaren RE. Gene therapy for retinal disease. Translational research: the journal of laboratory and clinical medicine. 2013; 161:241–254.10.1016/j.trsl.2012.12.007 [PubMed: 23305707]
49. Petrs-Silva H, Linden R. Advances in gene therapy technologies to treat retinitis pigmentosa. Clinical ophthalmology. 2014; 8:127–136.10.2147/OPTH.S38041 [PubMed: 24391438]
50. Dalkara D, Sahel JA. Gene therapy for inherited retinal degenerations. Comptes rendus biologies. 2014; 337:185–192.10.1016/j.crvi.2014.01.002 [PubMed: 24702845]
51. Surace EM, Auricchio A. Versatility of AAV vectors for retinal gene transfer. Vision research. 2008; 48:353–359.10.1016/j.visres.2007.07.027 [PubMed: 17923143]
52. Vandenberghe LH, Auricchio A. Novel adeno-associated viral vectors for retinal gene therapy. Gene Ther. 2012; 19:162–168.10.1038/gt.2011.151 [PubMed: 21993172]
53. Petrs-Silva H, Linden R. Advances in recombinant adeno-associated viral vectors for gene delivery. Current gene therapy. 2013; 13:335–345. [PubMed: 24060313]
54. Sarra GM, et al. Kinetics of transgene expression in mouse retina following sub-retinal injection of recombinant adeno-associated virus. Vision research. 2002; 42:541–549. [PubMed: 11853771]
55. Vandenberghe LH, et al. AAV9 targets cone photoreceptors in the nonhuman primate retina. PloS one. 2013; 8:e53463.10.1371/journal.pone.0053463 [PubMed: 23382846]
56. Vandenberghe LH, et al. Dosage thresholds for AAV2 and AAV8 photoreceptor gene therapy in monkey. Science translational medicine. 2011; 3:88–ra54.10.1126/scitranslmed.3002103
57. Boye SE, et al. The human rhodopsin kinase promoter in an AAV5 vector confers rod- and cone-specific expression in the primate retina. Human gene therapy. 2012; 23:1101–1115.10.1089/hum.2012.125 [PubMed: 22845794]
58. Oesterhelt D. The structure and mechanism of the family of retinal proteins from halophilic archaea. Current opinion in structural biology. 1998; 8:489–500. [PubMed: 9729742]
59. Bi A, et al. Ectopic expression of a microbial-type rhodopsin restores visual responses in mice with photoreceptor degeneration. Neuron. 2006; 50:23–33.10.1016/j.neuron.2006.02.026 [PubMed: 16600853]
60. Cepko C. Neuroscience. Seeing the light of day. Science. 2010; 329:403–404.10.1126/science.1194086 [PubMed: 20651144]
61. Busskamp V, Picaud S, Sahel JA, Roska B. Optogenetic therapy for retinitis pigmentosa. Gene therapy. 2012; 19:169–175.10.1038/gt.2011.155 [PubMed: 21993174]
62. Komaromy AM, et al. Transient photoreceptor deconstruction by CNTF enhances rAAV-mediated cone functional rescue in late stage CNGB3-achromatopsia. Mol Ther. 2013; 21:1131–1141.10.1038/mt.2013.50 [PubMed: 23568263]
63. McGee Sanftner LH, Abel H, Hauswirth WW, Flannery JG. Glial cell line derived neurotrophic factor delays photoreceptor degeneration in a transgenic rat model of retinitis pigmentosa. Mol Ther. 2001; 4:622–629.10.1006/mthe.2001.0498 [PubMed: 11735347]
64. Lau D, et al. Retinal degeneration is slowed in transgenic rats by AAV-mediated delivery of FGF-2. Invest Ophthalmol Vis Sci. 2000; 41:3622–3633. [PubMed: 11006261]
65. Buch PK, et al. In contrast to AAV-mediated Cntf expression, AAV-mediated Gdnf expression enhances gene replacement therapy in rodent models of retinal degeneration. Mol Ther. 2006; 14:700–709.10.1016/j.ymthe.2006.05.019 [PubMed: 16872907]

66. Liang FQ, et al. Long-term protection of retinal structure but not function using RAAV.CNTF in animal models of retinitis pigmentosa. Mol Ther. 2001; 4:461–472.10.1006/mthe.2001.0473 [PubMed: 11708883]

67. Bok D, et al. Effects of adeno-associated virus-vectored ciliary neurotrophic factor on retinal structure and function in mice with a P216L rds/peripherin mutation. Exp Eye Res. 2002; 74:719–735. [PubMed: 12126945]

68. Birch DG, Weleber RG, Duncan JL, Jaffe GJ, Tao W. Randomized trial of ciliary neurotrophic factor delivered by encapsulated cell intraocular implants for retinitis pigmentosa. Am J Ophthalmol. 2013; 156:283–292. e281. 10.1016/j.ajo.2013.03.021 [PubMed: 23668681]

69. Bennett J, et al. AAV2 gene therapy readministration in three adults with congenital blindness. Science translational medicine. 2012; 4:120–ra115.10.1126/scitranslmed.3002865

70. Hellstrom M, et al. Cellular tropism and transduction properties of seven adeno-associated viral vector serotypes in adult retina after intravitreal injection. Gene therapy. 2009; 16:521–532.10.1038/gt.2008.178 [PubMed: 19092858]

71. Yin L, et al. Intravitreal injection of AAV2 transduces macaque inner retina. Invest Ophthalmol Vis Sci. 2011; 52:2775–2783.10.1167/iovs.10-6250 [PubMed: 21310920]

72. Dalkara D, et al. In vivo-directed evolution of a new adeno-associated virus for therapeutic outer retinal gene delivery from the vitreous. Science translational medicine. 2013; 5:189–ra176.10.1126/scitranslmed.3005708

73. Klimczak RR, Koerber JT, Dalkara D, Flannery JG, Schaffer DV. A novel adeno-associated viral variant for efficient and selective intravitreal transduction of rat Muller cells. PloS one. 2009; 4:e7467.10.1371/journal.pone.0007467 [PubMed: 19826483]

74. Byrne LC, et al. Retinoschisin gene therapy in photoreceptors, Muller glia or all retinal cells in the Rs1h-/- mouse. Gene therapy. 201410.1038/gt.2014.31

75. Kay CN, et al. Targeting photoreceptors via intravitreal delivery using novel, capsid-mutated AAV vectors. PloS one. 2013; 8:e62097.10.1371/journal.pone.0062097 [PubMed: 23637972]

76. Srivastava A, Lusby EW, Berns KI. Nucleotide sequence and organization of the adeno-associated virus 2 genome. J Virol. 1983; 45:555–564. [PubMed: 6300419]

77. Yang J, et al. Concatamerization of adeno-associated virus circular genomes occurs through intermolecular recombination. J Virol. 1999; 73:9468–9477. [PubMed: 10516055]

78. Yan Z, Zhang Y, Duan D, Engelhardt JF. Trans-splicing vectors expand the utility of adeno-associated virus for gene therapy. Proc Natl Acad Sci U S A. 2000; 97:6716–6721. [PubMed: 10841568]

79. Duan D, Yue Y, Engelhardt JF. Expanding AAV packaging capacity with trans-splicing or overlapping vectors: a quantitative comparison. Mol Ther. 2001; 4:383–391.10.1006/mthe.2001.0456 [PubMed: 11592843]

80. Koo T, Popplewell L, Athanasopoulos T, Dickson G. Triple trans-splicing adeno-associated virus vectors capable of transferring the coding sequence for full-length dystrophin protein into dystrophic mice. Hum Gene Ther. 2014; 25:98–108.10.1089/hum.2013.164 [PubMed: 24191945]

81. Ghosh A, Yue Y, Lai Y, Duan D. A hybrid vector system expands adeno-associated viral vector packaging capacity in a transgene-independent manner. Mol Ther. 2008; 16:124–130.10.1038/sj.mt.6300322 [PubMed: 17984978]

82. Zhang Y, et al. Dual AAV therapy ameliorates exercise-induced muscle injury and functional ischemia in murine models of Duchenne muscular dystrophy. Hum Mol Genet. 2013; 22:3720–3729.10.1093/hmg/ddt224 [PubMed: 23681067]

83. Zhang Y, Duan D. Novel mini-dystrophin gene dual adeno-associated virus vectors restore neuronal nitric oxide synthase expression at the sarcolemma. Hum Gene Ther. 2012; 23:98–103.10.1089/hum.2011.131 [PubMed: 21933029]

84. Lostal W, et al. Efficient recovery of dysferlin deficiency by dual adeno-associated vector-mediated gene transfer. Hum Mol Genet. 2010; 19:1897–1907.10.1093/hmg/ddq065 [PubMed: 20154340]

85. Grose WE, et al. Homologous recombination mediates functional recovery of dysferlin deficiency following AAV5 gene transfer. PLoS One. 2012; 7:e39233.10.1371/journal.pone.0039233 [PubMed: 22720081]

86. Lopes VS, et al. Retinal gene therapy with a large MYO7A cDNA using adeno-associated virus. Gene Ther. 2013; 20:824–833.10.1038/gt.2013.3 [PubMed: 23344065]

87. Dyka FM, Boye SL, Chiodo VA, Hauswirth WW, Boye SE. Dual Adeno-Associated Virus Vectors Result in Efficient In Vitro and In Vivo Expression of an Oversized Gene, MYO7A. Hum Gene Ther Methods. 2014; 25:166–177.10.1089/hgtb.2013.212 [PubMed: 24568220]

88. Colella P, et al. Efficient gene delivery to the cone-enriched pig retina by dual AAV vectors. Gene Ther. 2014; 21:450–456.10.1038/gt.2014.8 [PubMed: 24572793]

89. Allocca M, et al. Serotype-dependent packaging of large genes in adeno-associated viral vectors results in effective gene delivery in mice. J Clin Invest. 2008; 118:1955–1964.10.1172/JCI34316 [PubMed: 18414684]

90. Dong B, Nakai H, Xiao W. Characterization of genome integrity for oversized recombinant AAV vector. Mol Ther. 2010; 18:87–92.10.1038/mt.2009.258 [PubMed: 19904236]

91. Lai Y, Yue Y, Duan D. Evidence for the failure of adeno-associated virus serotype 5 to package a viral genome > or = 8.2 kb. Mol Ther. 2010; 18:75–79.10.1038/mt.2009.256 [PubMed: 19904238]

92. Kapranov P, et al. Native molecular state of adeno-associated viral vectors revealed by singlemolecule sequencing. Hum Gene Ther. 2012; 23:46–55.10.1089/hum.2011.160 [PubMed: 21875357]

93. Trapani I, et al. Effective delivery of large genes to the retina by dual AAV vectors. EMBO Mol Med. 2014; 6:194–211.10.1002/emmm.201302948 [PubMed: 24150896]

94. Maclachlan TK, et al. Preclinical safety evaluation of AAV2-sFLT01- a gene therapy for age-related macular degeneration. Mol Ther. 2011; 19:326–334.10.1038/mt.2010.258 [PubMed: 21119620]

95. Cheng Y, et al. Prevalence of neutralizing factors against adeno-associated virus types 2 in age-related macular degeneration and polypoidal choroidal vasculopathy. Curr Gene Ther. 2013; 13:182–188. [PubMed: 23590636]

96. Bischof D, Cornetta K. Flexibility in cell targeting by pseudotyping lentiviral vectors. Methods Mol Biol. 2010; 614:53–68.10.1007/978-1-60761-533-0_3 [PubMed: 20225035]

97. Dunaief JL, et al. Retroviral gene transfer into retinal pigment epithelial cells followed by transplantation into rat retina. Hum Gene Ther. 1995; 6:1225–1229.10.1089/hum.19956.9-1225 [PubMed: 8527481]

98. Kido M, et al. Use of a retroviral vector with an internal opsin promoter to direct gene expression to retinal photoreceptor cells. Curr Eye Res. 1996; 15:833–844. [PubMed: 8921226]

99. Blomer U, Naldini L, Verma IM, Trono D, Gage FH. Applications of gene therapy to the CNS. Hum Mol Genet. 1996; 5 Spec:1397–1404. [PubMed: 8875243]

100. Bainbridge JW, et al. In vivo gene transfer to the mouse eye using an HIV-based lentiviral vector; efficient long-term transduction of corneal endothelium and retinal pigment epithelium. Gene Ther. 2001; 8:1665–1668.10.1038/sj.gt.3301574 [PubMed: 11895005]

101. Lipinski DM, et al. Vesicular stomatitis virus glycoprotein- and Venezuelan equine encephalitis virus-derived glycoprotein-pseudotyped lentivirus vectors differentially transduce corneal endothelium, trabecular meshwork, and human photoreceptors. Hum Gene Ther. 2014; 25:50–62.10.1089/hum.2013.009 [PubMed: 24125177]

102. Kostic C, et al. Activity analysis of housekeeping promoters using self-inactivating lentiviral vector delivery into the mouse retina. Gene Ther. 2003; 10:818–821.10.1038/sj.gt.3301948 [PubMed: 12704422]

103. Gruter O, et al. Lentiviral vector-mediated gene transfer in adult mouse photoreceptors is impaired by the presence of a physical barrier. Gene Ther. 2005; 12:942–947.10.1038/sj.gt.3302485 [PubMed: 15772686]

104. Kompella UB, Amrite AC, Pacha Ravi R, Durazo SA. Nanomedicines for back of the eye drug delivery, gene delivery, and imaging. Prog Retin Eye Res. 2013; 36:172–198.10.1016/j.preteyeres.2013.04.001 [PubMed: 23603534]

105. del Pozo-Rodriguez A, Delgado D, Gascon AR, Solinis MA. Lipid nanoparticles as drug/gene delivery systems to the retina. J Ocul Pharmacol Ther. 2013; 29:173–188.10.1089/jop.2012.0128 [PubMed: 23286300]

106. Han Z, et al. Comparative analysis of DNA nanoparticles and AAVs for ocular gene delivery. PLoS One. 2012; 7:e52189.10.1371/journal.pone.0052189 [PubMed: 23272225]

107. Koirala A, et al. Persistence of non-viral vector mediated RPE65 expression: case for viability as a gene transfer therapy for RPE-based diseases. J Control Release. 2013; 172:745–752.10.1016/j.jconrel.2013.08.299 [PubMed: 24035979]

108. Koirala A, Conley SM, Naash MI. A review of therapeutic prospects of non-viral gene therapy in the retinal pigment epithelium. Biomaterials. 2013; 34:7158–7167.10.1016/j.biomaterials.2013.06.002 [PubMed: 23796578]

109. Tamboli V, Mishra GP, Mitrat AK. Polymeric vectors for ocular gene delivery. Ther Deliv. 2011; 2:523–536.10.4155/tde.11.20 [PubMed: 21858246]

110. Diebold Y, Calonge M. Applications of nanoparticles in ophthalmology. Prog Retin Eye Res. 2010; 29:596–609.10.1016/j.preteyeres.2010.08.002 [PubMed: 20826225]

111. O'Reilly M, et al. Gene therapy for rare diseases: summary of a National Institutes of Health workshop, September 13, 2012. Hum Gene Ther. 2013; 24:355–362.10.1089/hum.2013.064 [PubMed: 23517518]

Table 1

Currently active clinical trials for inherited retinal dystrophies and age-related macular degeneration

Active Clinical Trials								
Disease	**Gene**	**Coded protein**	**Expression**	**Frequency**	**Vector platform**	**Delivery**	**Locations**	**ClinicalTrials.gov ID**
LCA2	*RPE65*	RPE-specific 65kDa protein	RPE	1:1,000,000	AAV2/2	SR	USA, UK, Israel	NCT00999609; NCT00749957; NCT01208389; NCT00516477; NCT00643747; NCT00481546; NCT00821340
Choroideremia	*CHM*	Rab Escort Protein 1 (REP-1)	ubiquitous	1:50,000	AAV2/2	SR	UK, Canada	NCT01461213; NCT02077361
Wet AMD	*FLT1*	FMS-like tyrosine kinase 1	soluble	1:2,000	AAV2/2	SR or IVT	Australia, USA	NCT01494805; NCT01024998
	PLG and COL18A1	Angiostatin and endostatin	soluble	1:2,000	EAIV lentivirusr	SR	USA	NCT01301443;NCT01678872
USH1B	*MYO7A*	Myosin VIIa	PR	1:60,000	EAIV lentivirus	SR	USA, France	NCT01505062; NCT02065011
Stargardt	*ABCA4*	PR-specific adenosine triphosphate-binding cassette transporter	PR	1:8-10,000	EAIV lentivirus	SR	USA, France	NCT01367444; NCT01736592
Leber hereditary optic neuropathy	*ND4*	NADH dehydrogenase	RGC	1:30-50,000	AAV2/2	SR	China, France	NCT01267422; NCT02064569
ArRP	*MERTK*	Mer receptor tyrosine kinase	RPE	Very rare	AAV2/2	SR	Saudi Arabia	NCT01482195

Abbreviations: LCA, Leber Congenital Amarousis; USH1B, Usher syndrome type 1b; ArRP, Autosomal recessive Retinitis Pigmentosa;; RPE, Retinal Pigment Epithelium, PR, Photoreceptors; RGC, Retinal Ganglion Cells; SR, Subretinal; IVT, Intravitreal