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REVIEW



# Ocular gene therapy for choroideremia: clinical trials and future perspectives

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**ABSTRACT**

**Introduction**: Gene therapy offers the potential for targeted replacement of single gene defects in inherited retinal degenerations.

**Areas covered**: Choroideremia is an X-linked blinding retinal disease resulting from deficiency of the *CHM* gene product, REP1. The disease represents an ideal target for retinal gene therapy, as it is readily diagnosed in the clinic, relatively homogenous in phenotype and slow progressing, thereby providing a wide therapeutic window for intervention. Ongoing clinical trials of retinal gene therapy for choroideremia using an adeno-associated viral vector have demonstrated safety and early efficacy. We review the clinical characteristics of the disease with a view to interpreting the findings of gene therapy clinical trials and discuss future directions.

**Expert commentary**: Choroideremia gene therapy has so far demonstrated good safety profile and early functional visual acuity gains in a proportion of trial participants, which appear to be sustained.

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AAV; adeno-associated virus; CHM; choroideremia; gene therapy; REP1

## 1. Introduction

### 1.1. Clinical characteristics of choroideremia

Choroideremia is an X-linked recessive retinal dystrophy caused by mutations within the *CHM* gene, which encodes Rab escort protein-1 (REP1), a protein involved in vesicular trafficking via the prenylation of Rab GTPases [1–3]. The majority of choroideremia patients have loss-of-function mutations [4,5], which cause progressive centripetal loss of retinal pigment epithelial (RPE) cells. The loss of photoreceptors appears to predominantly occur secondary to loss of the underlying RPE support, although some degree of independent photoreceptor loss may also occur [6–8]. Poor night vision in childhood is a common feature of the disease, which is indicative of impaired rod photoreceptor function. The nyctalopia may pass unnoticed by both the child and parents until chance events, such as trips to the cinema or countryside at night. In contrast, the cone photoreceptors appear to be less severely affected in choroideremia as most patients retain normal foveal visual acuity until the end stages of disease [9,10]. This may be explained by the existence of Müller cell-mediated alternative visual cycle pathway for the cones, which is independent of the RPE [11]. However, adaptive optics retinal imaging suggests that the cone density in the parafoveal region (at 300–600 µm eccentricity) is somewhat reduced even in early choroideremia, which indicates either a developmental abnormality or low-grade cone dysfunction [12].

Choroideremia was originally named after the prominent scleral vasculature visible on fundal examination, suggestive of choroidal atrophy [13]. However, the disease pathogenesis arises mainly from RPE and photoreceptor degeneration. Choroidal atrophy, which lags behind the degeneration of the overlying structures, likely occurs as a secondary event, possibly as part of a homeostatic response to reduced local oxygen requirement [14,15]. Unlike most forms of retinitis pigmentosa (RP), narrowing of the retinal vessels and waxy pallor of the optic disk are not features of choroideremia, suggesting that the inner retinal cells are generally well preserved. The characteristic fundal appearance, X-linked family history, small size of the *CHM* coding DNA sequence (1.9 kb), and long therapeutic window due to slow disease progression make choroideremia an ideal disease target for gene replacement therapy. Preclinical development of gene therapy for choroideremia has occurred over a decade, including proof-of-principle experiments in animal models [16,17] and cell models [18–21]. Here, we focus on the choroideremia gene therapy trials using the adeno-associated viral (AAV) vector to deliver normal human *CHM* complementary DNA (or transgene) into the diseased retina, which have demonstrated safety and early efficacy.

### 1.2. Clinical diagnosis and patient selection

While the fundal appearance of choroideremia is highly characteristic (Figure 1), a few rare retinal dystrophies may mimic the phenotype, in particular, carriers of dominant mutations in *RPE65* (chromosome 1) [22,23], Oliver McFarlane syndrome (*PNPLA6*, chromosome 19) [24], and gyrate atrophy (*OAT*, chromosome 10) [25]. The phenotypic similarity between choroideremia and dominant *RPE65*-mediated RP is pertinent since the *RPE65* gene is solely expressed in the RPE and absent in the choroid. This adds further support to choroideremia being

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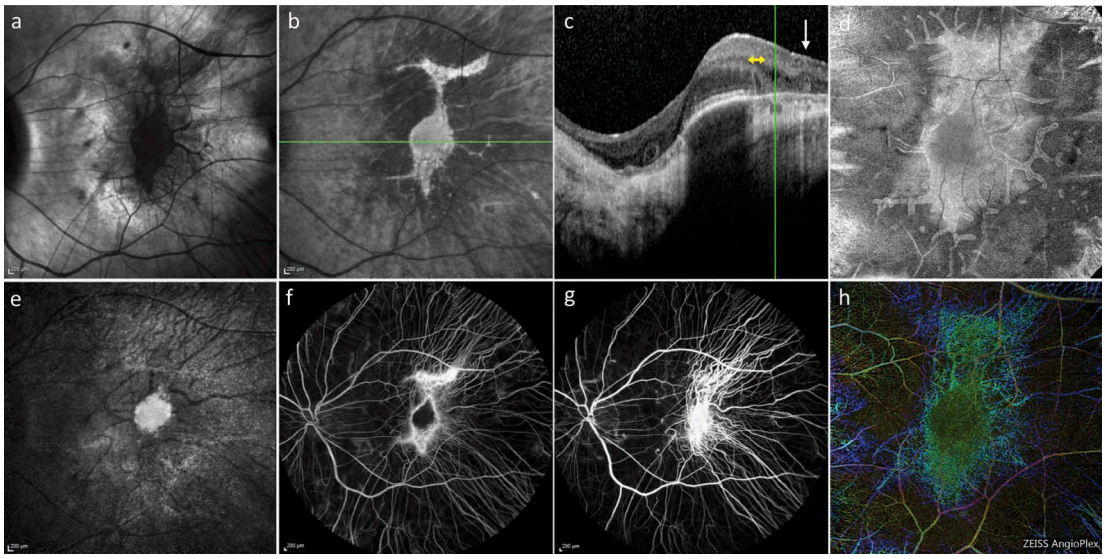


Figure 1. Multimodal retinal imaging in choroideremia. (A) Infra-red confocal scanning laser ophthalmoscopy (cSLO). (B) Blue-light (488 nm) fundus autofluorescence showing an island of preserved RPE. (C) Spectrial domain optical coherence tomography (SD-OCT). (D) En-face OCT. (E) Near infra-red (805 nm) autofluorescence (NIR-AF) indicating an area of preserved melanin pigment. (F) Fluorescein angiography. (G) Indocyanin green angiography. (H) Angio-OCT with color coded density map of choroidal vasculature underlying the residual retinal island. Full color available online.

primarily a disease of the RPE. While choroideremia is by far the most common (prevalence around 1 in 50,000 males) among the list of conditions above, observation of diffuse or patchy RPE mottling in the fundi of disease carriers within the family (e.g. the patient's mother) is very helpful to strengthen the diagnosis in the clinic [26]. For the purpose of genetic counseling and potential gene therapy enrollment, however, confirmation of the pathogenic mutation within the *CHM* gene through DNA sequencing remains essential. In our cohort of 79 choroideremia patients, causative mutations in *CHM* were found in 94% by exon sequencing [5]. Recently, a novel pathogenic mutation in the promoter region of *CHM* has been identified [27]. Despite this, there remain a small number of individuals in whom no *CHM* mutations could be found despite classic phenotype and a family history consistent with X-linked inheritance. In these cases, alternative molecular confirmation may be sought by measuring the levels of REP1 protein in patient-derived skin fibroblasts or blood [28].

Interestingly, while diffuse fine pattern RPE mottling is usually seen in female *CHM* carriers due to random X-inactivation, it is generally minimally progressive and not associated with any subjective visual deficit, although a small reduction in macular sensitivity is detectable through microperimetry [26]. The implication is that if gene therapy could achieve approximately 50% diffuse transduction of the RPE monolayer, clinical disease progression may be halted. Occasionally, however, female choroideremia carriers could be affected by patches of retinal degeneration, which expand gradually, thus becoming locally reminiscent of the male disease phenotype. These cases are thought to result from skewed X-inactivation during early retinal development. It could be inferred that if the gene therapy vector transduced the RPE cells non-uniformly, patchy disease progression may still occur. Therefore, both the proportion and distribution of target cell transduction during retinal gene therapy could ultimately influence the clinical efficacy of the treatment. This must be taken into account when interpreting the anatomical outcomes of choroideremia gene therapy.

A full assessment of the state of the retina in choroideremia is an important part of the workup toward retinal gene therapy. Over the past few years, several groups including ours have explored various modalities of retinal imaging with a view to identifying reliable anatomical markers of disease progression (Figure 1). Blue-light (488 nm wavelength) fundus autofluorescence (AF) imaging in choroideremia reveals the areas of surviving RPE. In the early stages of the disease, patches of RPE degeneration (indicated by loss of AF) appear in the mid to far peripheral retina. These dark patches gradually expand and coalesce, leading to near confluent peripheral visual field loss as the disease progresses over the first two decades of life. Toward the end of the second decade, a central residual 'island' of surviving and autofluorescent retina usually emerges. Since the area of the AF island undergoes gradual centripetal shrinkage with age and could be measured with a high degree of accuracy, it has emerged as a promising biomarker for monitoring disease progression. Our longitudinal study of a cohort of 31 choroideremia patients indicated that the area of the AF island undergoes exponential decay with a half-life of around 5.5 years [29]. While the retinal island usually demonstrates reduced sensitivity on microperimetry testing compared with normative data, the visual acuity is generally well maintained until the shrinkage encroaches upon the fovea, causing blindness around the fifth decade of life [9].

The structural integrity of the retina in choroideremia can be evaluated using high-resolution (e.g. spectral domain) optical coherence tomography (OCT). The ellipsoid zone (EZ) width seen on OCT cross-sections correlates closely with the horizontal dimensions of the AF island [14,30]. The quality of the EZ usually appears relatively normal centrally, but the EZ becomes somewhat mottled beyond an eccentricity of over 350 μm from the foveola [14], which coincides with morphological abnormalities

of the photoreceptors seen by adaptive optics scanning laser ophthalmoscopy [7,8,12]. The preserved EZ could be seen to be bracketed at either end by so-called transitional zones, which represent regions of sharp drop-off of the photoreceptor mosaic (Figure 1(c)). Around the edges of the EZ or AF island, tubulations are frequently observed, which are 'pseudo-dendritic' structures adjoining the main island of surviving outer retina when seen en-face (Figure 1(d)). They are thought to represent remodeling of the photoreceptors that have lost their outer segments after degeneration of the underlying RPE. Occasionally, the degeneration in choroideremia could be associated with foveal schisis or scleral pits resulting from severe retinal thinning [31] and may even be complicated by choroidal neovascularization [32]. These retinal changes need to be taken into account when selecting patients for gene therapy, because structural fragility of the retina could make subretinal injection of vector surgically challenging. Advanced foveal involvement may also be associated with a guarded visual prognosis.

Another mode of retinal imaging worth consideration is near-infrared (805 nm wavelength) AF (NIR-AF) (Figure 1(e)), which is distinct from standard blue-light AF imaging in detecting fluorescence arising from melanin pigment rather than lipofuscin. Unlike lipofuscin, which generally accumulates in disease and becomes absent after RPE cell death, as seen in choroideremia, melanin is 'naturally occurring' within RPE cells and its diminution may be an indicator of RPE cell dysfunction. Studies in Stargardt disease have indicated that changes in NIR-AF better correlated with the extent of photoreceptor degeneration seen by OCT and that it may provide a more sensitive biomarker for the extent of RPE disease than short-wavelength AF [33]. The relevance of NIR-AF in the evaluation of choroideremia remains to be fully explored, although our preliminary work has suggested some correlation between the level of parafoveal NIR-AF and macular sensitivity in the early stages of the disease [34].

## 2. Choroideremia gene therapy trials

### 2.1. Clinical trial outcomes

In 2014, we reported the interim (6 months) results of the first phase I/II clinical trial of gene therapy for choroideremia based in the United Kingdom (Gene Therapy for Blindness Caused by Choroideremia, *clinicaltrials.gov* ID: NCT01461213) [35]. This used an AAV serotype 2 vector carrying the human *CHM* transgene regulated by a ubiquitous cytomegalovirus-enhanced chicken β-actin (CAG) promoter, a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), and a modified bovine polyadenylation (polyA) signal. The trial design was open label, with a low-dose and a high-dose treatment group, and the worse eye was generally chosen with the primary intention of assessing safety. Initially, six patients underwent vitrectomy and subretinal injection of the AAV vector at the low dose ($1 \times 10^{10}$ viral genomes [vg] in 100 µl). In five patients, the intended full volume (100 µl) of vector was delivered into the subretinal space. In one patient, the retina proved to be highly adherent thus difficult to detach. This resulted in a reduced volume of vector (60 µl) being delivered into the subretinal space and possibly foveal stretch due to the excessive force required to detach the retina. All six patients received a 10-day course of systemic corticosteroid (prednisolone) in the perioperative period and no significant intraocular or systemic immune responses were detected. By 6 months, two of these patients, both with advanced disease associated with reduced visual acuity at baseline, gained 21 and 11 Early Treatment of Diabetic Retinopathy Score (ETDRS) letters in their best corrected visual acuity (BCVA), respectively. The other four patients, who had near normal levels of visual acuities (hence a 'ceiling effect') at baseline, recovered from the iatrogenic detachment of their entire central functioning retinal islands, including the fovea, to within one to three letters of their baseline visual acuity. While small fluctuations in ETDRS readings could occur in the short term, trends that emerge from multiple visual acuity measurements taken from treated versus control eyes over the long term would be more reliable indicators of a treatment effect. Subsequent follow-up of these patients showed maintenance of the visual acuity gains at 3.5 years, except in the patient that had surgical difficulty and reduced dose, who showed a 29-letter loss in the treated eye compared with 18-letter loss in the control eye [36]. Following the six patients that received the low dose, eight more patients went on to receive the high dose ($1 \times 10^{11}$ vg in 100 µl). The surgical technique was upgraded halfway through the trial to allow more gentle subretinal injection under footpedal control using the viscous fluid control port of the vitrectomy system [37,38]. This has been reported to enable successful delivery of viral vector with rapid recovery of baseline visual acuity and retinal sensitivity by 1 month [39]. The full results of this phase I/II clinical trial at its primary 2-year end point are anticipated.

Meanwhile, three other clinical trials using the higher dose ($1 \times 10^{11}$ vg) of the same AAV2.CAG.CHM.WPRE.polyA vector as the UK group were launched in Canada (Edmonton), USA (Miami), and Germany (Tübingen), each enrolling six participants (Table 1). Some of the early results of these trials have been discussed at the Association for Research in Vision and Ophthalmology Annual Meetings in 2016 and 2017; however, these initial reports in published abstracts should be approached with caution and the final outcomes at the 2-year trial end point are awaited. The Edmonton group reported immediate postoperative reduction in visual acuity in the treated eyes as expected following iatrogenic foveal detachment, which recovered to baseline by 3 months [40]. The Miami group reported their 6-month interim visual acuity data, which showed no significant difference in BCVA change between the six treated (1.7 ± 4.1 letters) and six control eyes (2.0 ± 3.1 letters) as a whole. An improvement of 10 letters by 1 month was observed in the treated eye of 1 participant, which was sustained up to 6 months [41]. These initial observations show safety, but longer term follow-up would be required to detect any potential difference in the rate of AF area loss. It should also be noted that comparison of the rate of AF area shrinkage between the treated and control eyes over time in choroideremia is not entirely straightforward (Figure 2). The reasons are mainly twofold. First, while natural history data would point to generally symmetric rates of AF area shrinkage between fellow eyes, some small differences in natural exponential decay half-life could be expected as choroideremia patients often present with one eye at a more advanced state of AF area loss than the other. Second,

Table 1. Summary of choroideremia gene therapy trials to date.

| Clinicaltrials.gov identifier | Phase | Design | Location | Enrollment | Vector | Start date |
|---|---|---|---|---|---|---|
| NCT01461213 | I/II | Low and high dose, open label | University of Oxford, UK | 14 | rAAV2.REP1 | October 2011 |
| NCT02341807 | I/II | Low and high dose, open label | Spark Therapeutics, USA | 15 | rAAV2.hCHM | January 2015 |
| NCT02077361 | I/II | Single dose, open label | University of Alberta, Canada | 6 | rAAV2.REP1 | April 2015 |
| NCT02553135 | I/II | Single dose, open label | University of Miami, USA | 6 | rAAV2.REP1 | September 2015 |
| NCT02671539 | I/II | Single dose, open label | University of Tübingen, Germany | 6 | rAAV2.REP1 | January 2016 |
| NCT02407678 | II | Randomized, open label | University of Oxford & Moorfields Eye Hospital, UK | 30 | rAAV2.REP1 | August 2016 |

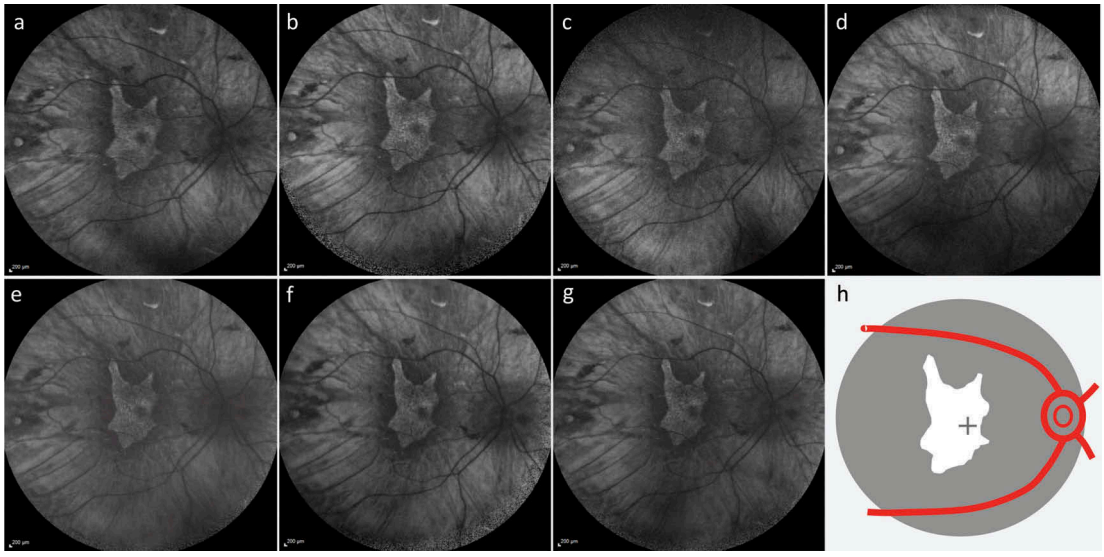


Figure 2. Gradual reduction in autofluorescence area over 6 years in an eye with choroideremia (A-G). (H) Schematic line drawing illustrating the outline of the island of residual autofluorescence in this eye in relation to the fovea (cross) and optic nerve head.

serial AF images of the same eye (e.g. taken using the Heidelberg Spectralis BluePeak AF Module, Heidelberg Engineering, Germany) can vary in terms of image focus, pixel density, edge distortion, and resolution, all of which can affect area measurements. Efforts are therefore being made by various groups to improve the algorithms for AF image registration to enable precise determination of small AF area changes [29].

As with the experience from the UK group published so far, choroideremia gene therapy has continued to demonstrate good safety profile at all the other centers. The Miami group noted two of their six patients with preexisting lamellar partial thickness retinal thinning developed a macular hole within the area of nonfunctional retina, which did not require treatment or adversely affect vision. Cataract formation may be expected following vitrectomy, which is a prerequisite to subretinal injection. One patient from the Miami trial developed worsening of a preexisting cataract following gene therapy. This was removed at 6 months post-gene therapy, which was associated with recovery of visual acuity to baseline level. The long-term follow-up data from our group showed that none of the six patients required cataract surgery before the 2-year trial end point, which may be partly related to the fact that all eyes were left fluid-filled (i.e. without any air or gas tamponade) at the end of surgery.

Based on the safety data from the phase I/II studies, a phase II clinical trial, REGENERATE (REP1 Gene Replacement Therapy for Choroideremia) (NTC01461213), began in the United Kingdom in 2015. This open-label single-dose ($1 \times 10^{11}$ vg) randomized trial aims to assess the efficacy of gene therapy in patients at an earlier stage of the disease who still have normal visual acuity and substantial areas of residual AF islands which may make area measurements more accurate.

Independent from the aforementioned choroideremia gene therapy trials which all use the same AAV vector construct containing WPRE, which enhances transgene expression [42], Spark Therapeutics Inc. (Philadelphia, USA) initiated a phase I/II trial of a similar AAV vector construct which does not contain WPRE in 2015 (NCT02341807). The results of this trial remain awaited.

### 2.2. Surgical approach and future improvements

Unlike standard medical therapies, minimally traumatic surgical delivery of AAV vector into the subretinal space is probably the single most important determinant of clinical outcome. Compared with intravitreal injection, the subretinal route of vector administration adopted in gene therapy for choroideremia is technically more challenging but has several advantages. Chiefly, it allows a higher concentration of vector to be placed in direct contact with the RPE and photoreceptors at the macula, thus maximizing the probability of viral transduction [43]. Since the vector enters a newly created potential space, the risk of triggering an acute immune response is also minimized. In

contrast, vitritis has been reported following intravitreal AAV delivery [44]. Iatrogenic detachment of the fovea in the diseased retina in choroideremia poses additional difficulties as the atrophic retina in the degenerate regions surrounding the residual island is highly adherent to the underlying Bruch's membrane, thereby strongly resisting the spread of a subretinal bleb. This increases the risk of excessive retinal stretch as the subretinal fluid builds up within a tense bleb, which could potentially cause damage to the fovea and reduce visual acuity, a particular concern when treating patients with normal visual acuity. We have previously described a two-step technique for subretinal gene therapy [38]. Briefly, a 41-ga Teflon-tipped cannula is first used to initiate a subretinal bleb with normal saline from the edge of the residual retinal island as seen on AF imaging. In a second step, the vector solution is injected with gentle pulses through the same retinotomy, slowly extending the bleb to involve the fovea in the process. Fine control of injection pressure can be achieved using a footpedal connected to the viscous fluid injection port of the vitrectomy machine and retinal stretch monitored in real-time using intraoperative OCT (Zeiss Rescan 7000, Carl Zeiss Meditec, Germany). When performing subretinal vector injection close to an area of thin retina (e.g. lamellar hole), one modification of this technique is to place a small bubble of heavy liquid (perfluorocarbon) over the area of thin retina so that the weight of the heavy liquid helps to counteract the stretching force of the subretinal injection over the most vulnerable part of the retina [45].

In advanced choroideremia, the centripetal shrinkage of the residual AF island leads to encroachment upon the fovea, which is associated with a relatively rapid decline in visual acuity. Evaluation of the structural integrity of the fovea is an important consideration prior to gene therapy as performing subretinal injection to a small AF island could cause foveal stretch or displacement of the surviving photoreceptors relative to the underlying RPE, thus risking a drop in visual acuity or shrinkage of the AF area in the short term. An additional issue arising from creation of a subretinal bleb is that the natural resorption pattern of the bleb could influence the level of AAV transduction across the detached area (Figure 3). Fick's law of diffusion dictates that the rate of absorption of the subretinal fluid ($\frac{\Delta q}{\Delta t}$, where $q$ = quantity of a solute and $t$ = time) would be proportional to the internal surface area of the bleb ($A$):

$$\frac{\Delta q}{\Delta t} = k \cdot A \cdot \Delta C$$

where $k$ is the diffusion coefficient, $A$ is the surface area for solute exchange, and $\Delta C$ is the concentration gradient. The internal surface area of a subretinal bleb could be estimated using the equation for the surface area of a hemisphere: $A = 3\pi r^2$, where $r$ is the radius of the bleb. Substituting this into Fick's law and rearranging for $r$ shows that $r$ would be expected to be proportion to the inverse of the square root of time:

$$r \propto \frac{1}{\sqrt{\Delta t}}$$

Therefore, as the retina reattaches, the rate of absorption of subretinal fluid would be expected to slow down at an accelerated pace, meaning that the RPE and retina at the center of the bleb would be exposed to the vector for much longer periods of time than that at the periphery of the bleb. This might mean that the cells at the fovea have the highest probability of successful viral transduction. Such a pattern of transduction would be advantageous in helping to rescue foveal function (e.g. causing improved visual acuity) but disadvantageous for the anatomical preservation of the edges of the retinal island because the periphery would receive the lowest vector dose.

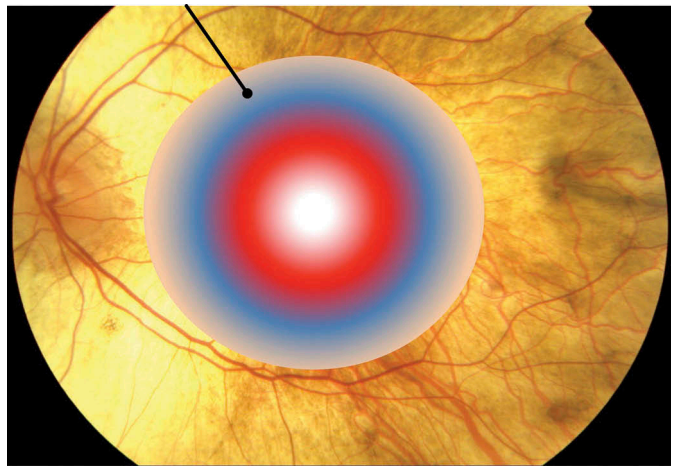

Figure 3. Schematic for the absorption of a subretinal bleb following imaginary injection of vector marked by the black line. Concentric rings illustrate the predicted shrinkage of a hypothetical circular subretinal bleb at set time intervals based on Fick's law of diffusion and surface area of a hemisphere. Due to the concave shape of the eye, the subretinal fluid will be deeper centrally and this will be the last point from which fluid will be reabsorbed as the retina reattaches. Hence, excluding any slight effects of gravity, retinal pigment epithelial cells lying in the central part of the bleb (white to red) are likely to have far more exposure time in contact with the vector solution than cells located in the periphery (blue to green). This is likely to result in a dose-profile gradient along the radial axis. Full color available online.

In the future, robotic assisted subretinal injection may help to further improve the safety and precision of gene therapy delivery beyond the level that could be achieved with manual surgery [46,47]. Preliminary testing of a vitreoretinal surgical robotic system coupled to the intraoperative OCT at our center has demonstrated safety in using the device to initiate epiretinal membrane or inner limiting membrane peeling, as well as in performing subretinal injection of tissue plasminogen activator (Alteplase) to treat submacular hemorrhage. Further development of the device could enable prolonged slow infusion of vector into the subretinal space, which could result in more accurate dosing and minimize the risk of iatrogenic retinal trauma.

### 2.3. Functional and anatomical end points

Given the differences in the level of visual impairment and rate of disease progression between different inherited retinal dystrophies, clinical trial end points need to be tailored to each disease. The early results emerging from gene therapy trials for choroideremia suggest that BCVA could be improved in a proportion of patients with significantly reduced visual acuity at baseline (between 20/20 and 20/200 Snellen acuity). So far, in the trials, eyes with acuity less than 20/200 have

been excluded on the basis that quantification of any BCVA changes would be difficult below this level and also that in such circumstances, the parafoveal region has often undergone irreversible RPE and photoreceptor cell loss, thus providing guarded visual prognosis.

For eyes with near-normal acuity, on the other hand, there would be a ceiling for potential visual acuity gain; therefore, secondary clinical end points would be needed. This is challenging as choroideremia is a very slow progressing disease. The two most promising secondary end points under investigation are mean retinal sensitivity as measured by microperimetry (a static threshold visual field test for the central macula region) and changes in residual AF area. While visual acuity is determined by the function of the central cone photoreceptors, retinal sensitivity would also be partly dependent on the rod function of the entire macula region. The disadvantages of microperimetry however are threefold. First, reliable testing requires stable foveal fixation from the patient, which is often difficult in advanced choroideremia [9]. Furthermore, any potential gain in retinal sensitivity from gene therapy must first offset any early reduction expected from the iatrogenic macula detachment and any late reduction resulting from cataract formation post-vitrectomy. These factors make the interpretation of retinal sensitivity data complex. In addition, subretinal AAV2 vector generally achieves higher transduction in RPE cells than photoreceptors (in part due to the phagocytic activity of RPE cells), as well as higher transduction in rods than cones [48,49]. Consequently, AAV2 gene therapy may be more effective at rescuing RPE cells (and rods, which depend on healthy RPE function) and less effective at boosting the sensitivity of cone photoreceptors. A corollary of microperimetry is that the inbuilt eye tracker function allows the point of fixation of the eye to be determined. In our phase I/II trial, one patient was noted to have shifted his preferred retinal locus from an area of degenerate fovea to a treated residual retinal island [35]. While this provides convincing evidence for clinical efficacy of the treatment, it is only applicable to the minority of patients who have lost foveal fixation.

The other promising secondary trial end point is the preservation of AF area, which represents an objective anatomical marker for RPE survival that could be compared between the treated and untreated eyes [12]. The caveat is that one makes the assumption that the AF area of fellow eyes of each individual should shrink at exactly the same rate. One must also make allowances for an initially accelerated AF area shrinkage as a result of iatrogenic retinal detachment in the treated eyes and any variability relating to the level of precision with which AF areas could be serially measured. We have studied the natural history of AF in choroideremia. Both cross-sectional and longitudinal studies indicate that the AF area undergoes exponential decay and estimated the annual percentage area reduction to be 7.7% and 11.8%, respectively [29,50]. The longitudinal AF area study suggested that although some variation in half-life exists between individuals, a reasonable degree of inter-eye symmetry exists in the rate of AF shrinkage. This means that it would be theoretically feasible to compare the AF area change in the treated eye against a predicted area shrinkage of around 20% in the untreated eye after 2–3 years. It seemed initially puzzling that despite apparent differences in AF island areas between the two eyes in some choroideremia patients, the AF area in both eyes underwent exponential decline with a similar half-life. A possible explanation is that the difference in AF areas between fellow eyes may arise more from difference in the time of onset of disease involving the posterior pole in each eye than small differences in the rate at which it progresses once the degeneration starts. Taken together, the evidence tentatively supports the use of fellow eye as control in clinical trials particularly when the baseline AF areas are symmetric between the two eyes. Alternatively, individuals can be observed for a period of time (e.g. multiple AF measurements taken over a year) to determine their baseline AF area half-life before a therapeutic intervention is given to one eye, because the percentage AF area reduction in the second year is predicted to match that in the first year.

Other potential clinical end points deserving of consideration are color perception and low light vision, since there have been multiple subjective reports of improved color vision from the clinical trials so far (e.g. improved distinction between different shades of green) and, to a lesser extent, improved night vision (e.g. seeing more stars in the night sky or greater confidence navigating in the dark). While deficits in color vision have been demonstrated in choroideremia [51], existing color perception tests can be difficult to perform in patients with grossly restricted visual fields, offer limited sensitivity, or have outputs that do not lend easily to quantitative analysis. While changes in color perception would reflect alterations of cone function only, changes in low light vision would be expected to reflect rod function. Various quantitative tests for low light visual acuity (e.g. low luminance BCVA) or retinal sensitivity (e.g. full field stimulus or scotopic microperimetry) are under validation as potentially new outcome measures for choroideremia gene therapy [52].

## 3. Future directions

Phase I/II clinical trials have so far established the safety of choroideremia gene therapy. Some clinical efficacy has also been demonstrated in terms of gains in visual acuity, which were sustained. A limiting factor to date has been that many patients enrolled have advanced disease with only small residual areas of surviving retina amenable to rescue. In the meantime, multicentered natural history studies involving large choroideremia patient cohorts have helped to build a detailed picture of the rate of visual acuity and anatomical decline in this rare disease [29,30]. The next stage of clinical investigation would take two main directions. First would be to investigate the efficacy of choroideremia gene therapy in terms of anatomical preservation in younger patients who have large areas of residual retina. In order to use the fellow eye as a reliable control, the treated and untreated eyes should ideally be symmetric in terms of visual acuity as well as residual AF area at baseline, thereby enabling randomization of treatment. Second, efficacy of gene therapy in terms of visual function improvement would need to be formally tested in a pivotal trial with predefined end points, e.g. BCVA at 1 or 2 years. This might involve treatment of a cohort of patients with reduced visual acuity in a double-blind randomized sham-controlled trial. Given the complexity of the gene therapy surgery and the general anesthesia involved, complete

blinding of a patient to the type of treatment received will be challenging; however, single-blinding of the investigator evaluating the clinical outcomes can be instituted.

Various strategies to optimize AAV transgene expression exist which may make their way into retinal gene therapy trials in the future. Moreover, it would also be crucial to achieve the maximal proportion of target cell transduction, since disease progression may continue despite successful transduction of a proportion of RPE or photoreceptor cells. Improved transduction of photoreceptors could be achieved using alternative naturally occurring AAV serotypes, e.g. AAV8 and AAV5 [53]. In terms of vector design, one strategy is to engineer AAV vectors with improved affinity for the desired tissue target. This has been successful through a variety of techniques, including targeted capsid amino acid modifications, capsid shuffling, directed evolution, and random peptide library insertions, resulting in a whole host of AAV variants with unique attributes [54–60]. These enhanced vectors could potentially better transduce photoreceptors or better penetrate the inner limiting membrane, thus enabling intravitreal delivery of the AAV vector to treat diseases of the outer retina. Once the vector has entered the target cell, expression of the transgene may be enhanced by codon-optimization, which emulates human tissue-specific codon preferences while evading innate immune response to the codon bias of infectious organisms [61,62]. While simply achieving high level of transgene expression may be desirable in some diseases, obtaining a more regulated or endogenous level of gene expression may be necessary in other diseases, since overexpression of some proteins may produce toxic effects. Optimizing the levels of gene expression in the human poses a formidable challenge, as observations based on animal (even nonhuman primate) models may not be truly representative of the situation in man. Therefore, selection of the most appropriate promoter for the transgene in each retinal disease will continue to be explored in the future.

## 4. Conclusion

Gene replacement therapy has the potential to treat a range of blinding monogenic retinal dystrophies. Phase I/II multicentered choroideremia gene therapy trials have so far demonstrated good safety. Clinical efficacy has also been seen in the form of sustained visual acuity gains in some patients with advanced disease. Further confirmation of these early findings is awaited through randomized controlled phase II and III trials in the coming years. Evaluation of the clinical outcome of these trials requires in-depth understanding of the natural disease progression of this rare disorder. A growing body of deep-phenotyping and natural history studies in choroideremia has helped to identify key outcome measures for gene therapy trials aimed at preserving visual function and retinal anatomy. These primarily include BCVA, retinal sensitivity, and AF area but may also include low light visual function and color perception.

## 5. Expert commentary

Multiple proof-of-concept studies in animal and cell models have provided support for gene replacement therapy in choroideremia. Multicentered clinical trials of choroideremia gene therapy have so far yielded promising results indicating general safety of the investigational medicinal product as well as the sub-macular route of vector delivery.

These open-label studies are somewhat limited by their modest sample sizes, which means that some uncertainty remains over the most effective vector dose. Vector dosing is further complicated by variations in AF island sizes between choroideremia patients, as subretinal vector injections tend to be more difficult in advanced disease and smaller retinal islands may be able to retain smaller volumes of AAV vector. Small differences in the amount of retinal stretch, volume delivered into subretinal space, or amount of vector refluxed into the vitreous cavity could all affect the clinical outcome. The surgical technique might be partially standardized by incorporating intraoperative OCT and identical injection systems at all the study centers. Robot-assisted delivery could further enable standardization of injection pressure and volume in the future. While traditional pharmacokinetics of medicinal products dictates that a standard dose should apply to all patients, viral vector-based treatments such as AAV may interact with each individual differently due to polymorphisms in cell surface receptors and intracellular antiviral responses to AAV. Therefore, rather than rigidly sticking to a fixed vector dose and volume, experience from a large number of patients may reveal that a more flexible strategy might be adopted such that the dose may be titrated to the area of tissue being targeted and limited to avoid excessive retinal stretch or inflammatory reaction. In addition, while ocular gene therapy has the advantage of an internal control in the fellow eye, the treatment efficacy may be better assessed in a larger number of participants with randomization of vector dosing as well as an element of blinding in the trial design.

AAV-mediated retinal gene therapy may lead to differences in the level of transduction between the RPE, rods, and cones exposed to the same vector dose, as well as differences between the same cell types at different locations within the retina. While retinal degeneration in choroideremia primarily causes loss of the RPE, some independent degeneration of the photoreceptors may also occur based on the tissue-specific conditional *Chm* knockout model in the mouse and EZ mottling seen within the surviving AF islands [6,7]. Differences in surgical delivery of the viral vector into the subretinal space and the kinetics with which the subretinal bleb is absorbed may also affect the treatment effects on the fovea versus peripheral macula (Figure 3). In addition, differences in genetic polymorphisms between patients might result in individual differences in cell surface receptors and intra-cellular antiviral responses [63], which could have a significant impact on the level of AAV transduction and durability of transgene expression. Tests to assess the efficacy of gene therapy would therefore need to be interpreted with cell-type specificity and individual variability in mind. Therefore, clinical outcome measures for retinal gene therapy may need to be multimodal in order to gleam differential benefits between individuals, including AF imaging, which assesses RPE survival, BCVA and color perception tests, which assess cone function, and low-light visual function tests, which assess rod function.

## 6. Five-year view

The first ever AAV-based gene therapy, alipogene tiparvovec (Glybera, Uniqure, the Netherlands), was approved by the

European Medicines Agency (EMA) in 2012 for the treatment of familial lipoprotein lipase deficiency (LPLD). More recently, the Food and Drug Administration (FDA) has approved voretigene neparvovec-rzyl (LUXTURNA®, Spark Therapeutics Inc., USA) to treat Lebers congenital amaurosis (LCA) due to mutations in *RPE65* [64]. The latter represents the first retinal gene therapy to receive FDA approval and provides great impetus for similar AAV-based gene replacement approaches to treat other inherited retinal diseases. Interestingly, given the profound nature of visual impairment in *RPE65* LCA, the primary outcome measure employed was a validated visual navigation test rather than visual acuity. This represents a welcome recognition that simplistic standard functional visual tests such as BCVA may not be adequate to demonstrate the full benefits of novel therapies in RP patients with complex visual impairment.

The results of phase II and phase III trials of choroideremia gene therapy are anticipated within the next few years both from the Nightstar Therapeutics (a University of Oxford/Wellcome Trust spin-out company, UK) and Spark Therapeutics sponsored trials. Within the next 5 years, we could potentially also see the results of later stage clinical trials of gene therapy for other monogenic recessive retinal diseases, including X-linked RP (*RPGR*) [65,66], *MERTK* [67], achromatopsia (*CNGA3* and *CNGB3*), and Best's disease (*BEST1*). One of the limitations of the AAV vector is its limited cargo capacity (<4.7 kb). Dual-vector approaches have shown early promise in animal models to help overcome this by carrying overlapping gene fragments, which recombine in the target cell to form the whole coding sequence. This could allow the delivery of larger transgenes, such as *ABCA4* (Stargardt disease) and *MYO7A* (Usher 2A) (both over 6 kb) [68–71]. Another maturing technology is CRISPR/Cas9-based genome editing. This could potentially broaden the feasibility of gene therapy to treat autosomal dominant retinal dystrophies by targeted correction of DNA mutations [72].

While we have undoubtedly entered an exciting era of emerging gene therapies for retinal diseases, there will be fresh challenges ahead with regards to how the manufacturing infrastructure and developmental pipeline for such biologically complex medicinal products can be maintained and how the treatment will be best distributed within the evolving health-care system.

## Key issues

- Phase I clinical trial of gene replacement therapy for choroideremia has demonstrated good safety and signs of efficacy in terms of gains in visual acuity, which are sustained.
- A growing body of deep-phenotyping and natural history studies in choroideremia have helped to establish the key endpoints for gene therapy clinical trials aimed at preserving visual function and retinal anatomy. These include best-corrected visual acuity, retinal sensitivity and autofluorescence area.
- The results of phase II and III choroideremia gene therapy trials are expected within the next few years.

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## Declaration of interest

RE MacLaren is the scientific founder of Nightstar Therapeutics Inc. RE MacLaren is a consultant to Spark Therapeutics Inc. Neither companies had any role in the writing of this review article. The views expressed are those of the authors and not necessarily those of the Wellcome Trust, the National Health Service, the NIHR, or the UK Department of Health. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. One peer reviewer was a scientific director of a trial being run by Spark Therapeutics.

## References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Cremers FP, van de Pol DJ, van Kerkhoff LP, et al. Cloning of a gene that is rearranged in patients with choroideraemia. Nature. 1990;347:674–677.
•• **Identification of the gene underlying choroideremia.**
2. Seabra MC, Brown MS, Goldstein JL. Retinal degeneration in choroideremia: deficiency of rab geranylgeranyl transferase. Science. 1993;259:377–381.
•• **Identification of prenylation defect in choroideremia.**
3. van Bokhoven H, van den Hurk JAJM, Bogerd LPM, et al. Cloning and characterization of the human choroideremia gene. Hum Mol Genet. 1994;3:1041–1046.
4. van den Hurk JA, Schwartz M, van Bokhoven H, et al. Molecular basis of choroideremia (CHM): mutations involving the Rab escort protein-1 (REP-1) gene. Hum Mutat. 1997;9:110–117, Review.
5. Simunovic MP, Jolly JK, Xue K, et al. The spectrum of CHM gene mutations in choroideremia and their relationship to clinical phenotype. Invest Ophthalmol Vis Sci. 2016;57:6033–6039.
6. Tolmachova T, Anders R, Abrink M, et al. Independent degeneration of photoreceptors and retinal pigment epithelium in conditional knockout mouse models of choroideremia. J Clin Invest. 2006;116:386–394.
• **Evidence suggesting independent degeneration of the RPE and photoreceptors in mouse.**
7. Morgan JI, Han G, Klinman E, et al. High-resolution adaptive optics retinal imaging of cellular structure in choroideremia. Invest Ophthalmol Vis Sci. 2014;55:6381–6397.
8. Sun LW, Johnson RD, Williams V, et al. Multimodal imaging of photoreceptor structure in choroideremia. PLoS One. 2016;11:e0167526.
9. Jolly JK, Xue K, Edwards TL, et al. Characterizing the natural history of visual function in choroideremia using microperimetry and multimodal retinal imaging. Invest Ophthalmol Vis Sci. 2017;58:5575–5583.
10. Aleman TS, Han G, Serrano LW, et al. Natural history of the central structural abnormalities in choroideremia: a prospective cross-sectional study. Ophthalmology. 2017;124:359–373.
11. Wang JS, Kefalov VJ. The cone-specific visual cycle. Prog Retin Eye Res. 2011;30:115–128.
12. Nabholz N, Lorenzini MC, Bocquet B, et al. Clinical evaluation and cone alterations in choroideremia. Ophthalmology. 2016;123:1830–1832.
13. Mauthner L. Ein fall von choroideremie. Ber Naturwissensch-Med Ver Inssbruck. 1872;2:191–197.
• **Original description of choroideremia as a retinal dystrophy.**
14. Xue K, Oldani M, Jolly JK, et al. Correlation of optical coherence tomography and autofluorescence in the outer retina and choroid

of patients with choroideremia. Invest Ophthalmol Vis Sci. 2016;57:3674–3684.
15. Jain N, Jia Y, Gao SS, et al. Optical coherence tomography angiography in choroideremia: correlating choriocapillaris loss with overlying degeneration. JAMA Ophthalmol. 2016;134:697–702.
16. Tolmachova T, Tolmachov OE, Barnard AR, et al. Functional expression of Rab escort protein 1 following AAV2-mediated gene delivery in the retina of choroideremia mice and human cells ex vivo. J Mol Med (Berl). 2013;91:825–837.
•• **Proof-of-principle for choroideremia gene therapy.**
17. Black A, Vasireddy V, Chung DC, et al. Adeno-associated virus 8-mediated gene therapy for choroideremia: preclinical studies in in vitro and in vivo models. J Gene Med. 2014;16:122–130.
18. Anand V, Barral DC, Zeng Y, et al. Gene therapy for choroideremia: in vitro rescue mediated by recombinant adenovirus. Vision Res. 2003;43:919–926.
19. Vasireddy V, Mills JA, Gaddameedi R, et al. AAV-mediated gene therapy for choroideremia: preclinical studies in personalized models. PLoS One. 2013;8:e61396.
20. Cereso N, Pequignot MO, Robert L, et al. Proof of concept for AAV2/5-mediated gene therapy in iPSC-derived retinal pigment epithelium of a choroideremia patient. Mol Ther Methods Clin Dev. 2014;1:14011.
21. Duong TT, Vasireddy V, Ramachandran P, et al. Use of induced pluripotent stem cell models to probe the pathogenesis of choroideremia and to develop a potential treatment. Stem Cell Res. 2018;27:140–150.
22. Hull S, Mukherjee R, Holder GE, et al. The clinical features of retinal disease due to a dominant mutation in RPE65. Mol Vis. 2016;22:626–635.
23. Bowne SJ, Humphries MM, Sullivan LS, et al. A dominant mutation in RPE65 identified by whole-exome sequencing causes retinitis pigmentosa with choroidal involvement. Eur J Hum Genet. 2011;19:1074–1081.
24. Sampson JR, Tolmie JL, Cant JS. Oliver McFarlane syndrome: a 25-year follow-up. Am J Med Genet. 1989;34:199–201.
25. Hayasaka S, Shoji K, Kanno C, et al. Differential diagnosis of diffuse choroidal atrophies. Diffuse choriocapillaris atrophy, choroideremia, and gyrate atrophy of the choroid and retina. Retina. 1985;5:30–37.
26. Edwards TL, Groppe M, Jolly JK, et al. Correlation of retinal structure and function in choroideremia carriers. Ophthalmology. 2015;122:1274–1276.
27. Radziwon A, Arno G, Wheaton D K, et al. Single-base substitutions in the CHM promoter as a cause of choroideremia. Hum Mutat. 2017;38:704–715.
28. Furgoch MJ, Mewes-Arès J, Radziwon A, et al. Molecular genetic diagnostic techniques in choroideremia. Mol Vis. 2014;20:535–544.
29. Aylward JW, Xue K, Patrício MI, et al. Retinal degeneration in choroideremia follows an exponential decay function. Ophthalmology. 2018:S0161-6420(18)30245–8. DOI: 10.1016/j.ophtha.2018.02.004
• **Longitudinal study showing exponential decline in autofluorescent island area in choroideremia.**
30. Hariri AH, Velaga SB, Girach A, Natural History of the Progression of Choroideremia (NIGHT) Study Group; et al. . Measurement and reproducibility of preserved ellipsoid zone area and preserved retinal pigment epithelium area in eyes with choroideremia. Am J Ophthalmol. 2017;179:110–117.
31. Al-Qahtani AA, Ba-Ali S, Alabduljalil T, et al. Scleral pits in choroideremia: implications for retinal gene therapy. Retina. 2017. DOI:10.1097/IAE.0000000000001957.
32. Patel RC, Gao SS, Zhang M, et al. Optical coherence tomography angiography of choroidal neovascularisation in four inherited retinal dystrophies. Retina. 2016;36:2339–2347.
33. Duncker T, Marsiglia M, Lee W, et al. Correlations among near-infrared and short-wavelength autofluorescence and spectral-domain optical coherence tomography in recessive Stargardt disease. Invest Ophthalmol Vis Sci. 2014;55:8134–8143.
34. Salvetti AP, Birtel J, Xue K, et al. Near-infrared autofluorescence in choroideremia: anatomical and functional correlations. Proceedings of the ARVO Annual Meeting, May 7–11, 2017, Baltimore (MD). [cited 2018 Apr 17]. Available from: http://iovs.arvojournals.org/article.aspx?articleid=2641833&resultClick=1
35. MacLaren RE, Groppe M, Barnard AR, et al. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. Lancet. 2014;383:1129–1137.
•• **First human trial of gene therapy for choroideremia.**
36. Edwards TL, Jolly JK, Groppe M, et al. Visual acuity after retinal gene therapy for choroideremia. N Engl J Med. 2016;374:1996–1998.
•• **Sustained visual acuity gains following choroideremia gene therapy.**
37. Fischer MD, Hickey DG, Singh MS, et al. Evaluation of an optimized injection system for retinal gene therapy in human patients. Hum Gene Ther Methods. 2016;27:150–158.
38. Xue K, Groppe M, Salvetti AP, et al. Technique of retinal gene therapy: delivery of viral vector into the subretinal space. Eye (Lond). 2017;31:1308–1316.
• **Surgical technique for choroideremia gene therapy.**
39. Simunovic MP, Xue K, Jolly JK, et al. Structural and functional recovery following limited iatrogenic macular detachment for retinal gene therapy. JAMA Ophthalmol. 2017;135:234–241.
40. MacDonald IM, Dimopoulos I, Chan S, et al. Ocular gene therapy for choroideremia: the Alberta experience. Proceedings of the ARVO Annual Meeting, May 1-5, 2016, Seattle (WA). [cited 2018 Apr 17]. Available from: http://iovs.arvojournals.org/article.aspx?articleid=2560404&resultClick=1
41. Lam BL, Verriotto J, Gregori N, et al. Choroideremia gene therapy phase II clinical trial: 6-month results. Proceedings of the ARVO Annual Meeting, May 7–11, 2017, Baltimore (MD). [cited 2018 Apr 17]. Available from: http://iovs.arvojournals.org/article.aspx?articleid=2640714&resultClick=1
42. Patrício MI, Barnard AR, Orlans HO, et al. Inclusion of the woodchuck hepatitis virus posttranscriptional regulatory element enhances AAV2-driven transduction of mouse and human retina. Mol Ther Nucleic Acids. 2017;6:198–208.
43. Seitz IP, Michalakis S, Wilhelm B, et al. RD-CURE consortium. Superior retinal gene transfer and biodistribution profile of subretinal versus intravitreal delivery of AAV8 in Nonhuman primates. Invest Ophthalmol Vis Sci. 2017;58:5792–5801.
44. Guy J, Feuer WJ, Davis JL, et al. Gene therapy for Leber hereditary optic neuropathy: low- and medium-dose visual results. Ophthalmology. 2017;124:1621–1634.
45. Maguire AM, High KA, Auricchio A, et al. Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. Lancet. 2009;374:1597–1605.
• **Sustained visual improvement following gene therapy for RPE65 Lebers congenital amaurosis.**
46. de Smet MD, Stassen JM, Meenink TC, et al. Release of experimental retinal vein occlusions by direct intraluminal injection of ocriplasmin. Br J Ophthalmol. 2016;100:1742–1746.
47. Edwards TL, Xue K, Meenink HCM, et al. A first-in-man clinical trial assessing robotic surgery inside the human eye: the robotic retinal dissection device (R2D2) study. Nature Biomedical Engineering; 2018. p. 1–15. (manuscript accepted for publication).
48. Yang GS, Schmidt M, Yan Z, et al. Virus-mediated transduction of murine retina with adeno-associated virus: effects of viral capsid and genome size. J Virol. 2002;76:7651–7660.
49. Vandenberghe LH, Bell P, Maguire AM, et al. Dosage thresholds for AAV2 and AAV8 photoreceptor gene therapy in monkey. Sci Transl Med. 2011;3:88ra54.
• **AAV vector selection and dosing for retinal gene therapy.**
50. Jolly JK, Edwards TL, Moules J, et al. A qualitative and quantitative assessment of fundus autofluorescence patterns in patients with choroideremia. Invest Ophthalmol Vis Sci. 2016;57:4498–4503.
51. Jolly JK, Groppe M, Birks J, et al. Functional defects in color vision in patients with choroideremia. Am J Ophthalmol. 2015;160:822–31.e3.
52. Dimopoulos IS, Freund PR, Knowles JA, et al. The natural history of full-field stimulus threshold decline in choroideremia. Retina. 2017. DOI:10.1097/IAE.0000000000001764.

53. Allocca M, Mussolino C, Garcia-Hoyos M, et al. Novel adeno-associated virus serotypes efficiently transduce murine photoreceptors. J Virol. 2007;81:11372–11380.
54. Zhong L, Li B, Mah CS, et al. Next generation of adeno-associated virus 2 vectors: point mutations in tyrosines lead to high-efficiency transduction at lower doses. Proc Natl Acad Sci U S A. 2008;105:7827–7832.
55. Bartel MA, Weinstein JR, Schaffer DV. Directed evolution of novel adeno-associated viruses for therapeutic gene delivery. Gene Ther. 2012;19:694–700.
56. Dalkara D, Byrne LC, Klimczak RR, et al. In vivo-directed evolution of a new adeno-associated virus for therapeutic outer retinal gene delivery from the vitreous. Sci Transl Med. 2013;5:189ra76.
57. Kotterman MA, Schaffer DV. Engineering adeno-associated viruses for clinical gene therapy. Nat Rev Genet. 2014;15:445–451.
58. Büning H, Huber A, Zhang L, et al. Engineering the AAV capsid to optimize vector-host-interactions. Curr Opin Pharmacol. 2015;24:94–104.
59. Zinn E, Pacouret S, Khaychuk V, et al. In silico reconstruction of the viral evolutionary lineage yields a potent gene therapy vector. Cell Rep. 2015;12:1056–1068.
60. Hickey DG, Edwards TL, Barnard AR, et al. Tropism of engineered and evolved recombinant AAV serotypes in the rd1 mouse and ex vivo primate retina. Gene Ther. 2017;24:787–800.
61. Plotkin JB, Robins H, Levine AJ. Tissue-specific codon usage and the expression of human genes. Proc Natl Acad Sci U S A. 2004;101:12588–12591.
62. Quax TE, Claassens NJ, Söll D, et al. Codon bias as a means to fine-tune gene expression. Mol Cell. 2015;59:149–161.
63. Reichel FF, Dauletbekov DL, Klein R, et al. RD-CURE Consortium. AAV8 can induce innate and adaptive immune response in the primate eye. Mol Ther. 2017;25:2648–2660.
64. Russell S, Bennett J, Wellman JA, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with *RPE65*-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. Lancet. 2017;390:849–860.
• **Phase III trial of gene therapy for RPE65 Lebers congenital amaurosis leading to the first FDA approval of a retinal gene therapy.**
65. Wu Z, Hiriyanna S, Qian H, et al. A long-term efficacy study of gene replacement therapy for RPGR-associated retinal degeneration. Hum Mol Genet. 2015;24:3956–3970.
66. Fischer MD, McClements ME, Martinez-Fernandez de la Camara C, et al. Codon-optimized RPGR improves stability and efficacy of AAV8 gene therapy in two mouse models of X-linked retinitis pigmentosa. Mol Ther. 2017;25:1854–1865.
67. Ghazi NG, Abboud EB, Nowilaty SR, et al. Treatment of retinitis pigmentosa due to MERTK mutations by ocular subretinal injection of adeno-associated virus gene vector: results of a phase I trial. Hum Genet. 2016;135:327–343.
68. Dyka FM, Boye SL, Chiodo VA, et al. 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.
69. Colella P, Trapani I, Cesi G, et al. Efficient gene delivery to the cone-enriched pig retina by dual AAV vectors. Gene Ther. 2014;21:450–456.
70. Trapani I, Colella P, Sommella A, et al. Effective delivery of large genes to the retina by dual AAV vectors. EMBO Mol Med. 2014;6:194–211.
71. Trapani I, Toriello E, de Simone S, et al. Improved dual AAV vectors with reduced expression of truncated proteins are safe and effective in the retina of a mouse model of Stargardt disease. Hum Mol Genet. 2015;24:6811–6825.
72. Suzuki K, Tsunekawa Y, Hernandez-Benitez R, et al. In vivo genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. Nature. 2016;540:144–149.