Gene therapy for choroideremia using an adeno-associated viral vector encoding Rab escort protein 1: the REGENERATE open-label trial

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Scientific summary

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Scientific summary

Background

Choroideremia is an X-linked inherited retinal degeneration, affecting approximately 1 in 50,000 people, that begins in childhood with nyctalopia and loss of peripheral vision and gradually progresses to blindness in adulthood. Null mutations in the *CHM* gene cause a deficiency of Rab escort protein 1 (REP1), leading to degeneration of the retinal pigment epithelium (RPE), followed by secondary degeneration of photoreceptors and the choroid. As the central cone photoreceptors are generally preserved until the late stages of choroideremia, due to the centripetal nature of the degeneration, patients usually retain good visual acuity until degeneration encroaches on the fovea.

Gene therapy, a procedure whereby a disease is treated at the genetic level by intracellular delivery of a therapeutic transgene, is an appealing strategy for treating choroideremia. Furthermore, the eye is an attractive site for gene therapy for several reasons. Surgical access is relatively straightforward and gene therapy products can be administered directly to cells in the outer retina (photoreceptors and RPE) by subretinal injection. Potential adverse events can be detected and monitored directly via ocular examination. Therapeutic outcomes can be measured directly using non-invasive assessments of visual function and retinal anatomy, with an untreated contralateral eye available as a control. The risk of systemic immune reactions is reduced by the anatomical compartmentalisation of the eye and the immunological privilege provided by the blood–retina barrier. Moreover, the small tissue volume of the subretinal space means that the therapeutic dose required to treat the retina is several thousand times lower in comparison with doses required for treatment of other organs, further reducing the risk of systemic adverse reactions.

Most gene therapies developed thus far as potential treatments for inherited retinal diseases have utilised recombinant adeno-associated virus (AAV) particles as vectors to deliver therapeutic transgenes to target cells in the retina. This gene therapy for choroideremia uses an AAV serotype 2 (AAV2) vector encoding human REP1 protein (AAV2-REP1) that was first tested in human subjects in a phase I/II safety and dose escalation study (NCT01461213) at Oxford (UK). A cohort of six participants was initially treated with a dose of up to 1×10^{10} vector particles administered subretinally in one eye, followed by a cohort of eight participants treated with a higher dose of up to 1×10^{11} vector particles. Two serious adverse events (SAEs) occurred during the clinical trial, comprising a case of retinal thinning due to surgical complications and a case of postoperative inflammation, but otherwise the study data confirmed the safety and possible efficacy of the AAV2-REP1 vector for treatment for choroideremia (Xue K, Jolly JK, Barnard AR, Rudenko A, Salvetti AP, Patrício MI, *et al*. Beneficial effects on vision in patients undergoing retinal gene therapy for choroideremia. *Nat Med* 2018;**24**:1507–12).

An additional 18 choroideremia patients were subsequently treated with a dose of up to 1×10^{11} particles of the same AAV2-REP1 vector administered subretinally in one eye – 6 participants in a phase I/II study (NCT02077361) at Edmonton (Canada), 6 participants in a phase II study (NCT02553135) at Miami (USA) and 6 participants in a phase II study (NCT02671539) at Tübingen (Germany). These studies showed that the AAV2-REP1 vector was generally well tolerated, with a subset of participants achieving clinically significant improvements in best corrected visual acuity (BCVA). One SAE involving a case of postoperative inflammation was reported in the Edmonton study (Dimopoulos IS, Hoang SC, Radziwon A, Binczyk NM, Seabra MC, MacLaren RE, *et al*. Two-year results after AAV2-mediated gene therapy for choroideremia: the Alberta experience. *Am J Ophthalmol* 2018;**193**:130–42). There were no cases of postoperative inflammation reported in the 12 choroideremia patients treated in Tübingen and Miami. In the Tübingen study, a pre-existing degenerative macular hole opened up in one of the participants during surgery but subsequently closed spontaneously (Fischer MD, Ochakovski GA, Beier B, Seitz IP, Vaheb Y, Kortuem C, *et al*. Efficacy and safety of retinal gene therapy using adeno-associated

virus vector for patients with choroideremia: a randomized clinical trial. *JAMA Ophthalmol* 2019;**137**:1247–54). In the Miami study, two participants developed an atrophic retinal hole in a non-functioning macular area (Lam BL, Davis JL, Gregori NZ, MacLaren RE, Girach A, Verriotto JD, *et al*. Choroideremia gene therapy phase 2 clinical trial: 24-month results. *Am J Ophthalmol* 2019;**197**:65–73).

In view of these satisfactory clinical outcomes, which indicated that gene therapy for choroideremia using the AAV2-REP1 vector demonstrated a good safety profile and sustained gains in BCVA in a proportion of trial participants, the REGENERATE (REP1 gene replacement therapy) phase II study (NCT02407678) was initiated to assess the efficacy of gene therapy (dose of up to 1×10^{11} particles of the AAV2-REP1 vector administered subretinally in one eye) in 30 choroideremia patients at an earlier stage of the disease and therefore still possessing substantial areas of surviving retinal tissue, thereby facilitating the measurement of area changes in order to assess the effect of gene therapy on retinal deterioration.

Objectives

The aim of the REGENERATE study was to investigate the efficacy and safety of a single subretinal injection of AAV2-REP1 in participants with a confirmed diagnosis of choroideremia over an assessment period of 24 months post treatment.

The primary safety-related outcome was change from baseline in BCVA in treated eyes at 24 months post treatment, with prospective efficacy evaluated by comparative change from baseline in BCVA in treated and untreated contralateral (control) eyes. Secondary outcomes included comparative change from baseline in mean retinal sensitivity (microperimetry) and retinal anatomy (area of autofluorescence) in treated and control eyes at 24 months post treatment, as well as change from baseline in safety-related immunological and physiological indicators.

Methods

Study design

The REGENERATE study is an open-label Phase II clinical trial investigating the efficacy and safety of AAV2-REP1 vector-mediated gene therapy for treatment of choroideremia.

In contrast to other interventional studies, the REGENERATE study recruited choroideremia patients at an earlier stage of the disease and therefore still possessing substantial areas of surviving retinal tissue. The intention behind this study design was to facilitate the measurement of changes in the area of surviving RPE (determined by fundus autofluorescence) in order to assess the effect of gene therapy on retinal deterioration.

Whereas in changes in BCVA and other measures of visual function in the treated eye could be compared against baseline values, assessment of the anatomical rate of degeneration ideally required the untreated contralateral eye to be used as an internal control. This condition required, in turn, the inclusion of participants having a fairly symmetrical disease. In consequence, randomisation of treatment of one eye or the other was also required to avoid selection bias.

Sample size

As this was an exploratory study to assess a new end point (comparative change from baseline in the area of surviving RPE in the treated and control eyes, determined by fundus autofluorescence), there was no predetermined power calculation to determine the number of trial participants. Instead, data from this trial will be used for future power calculations. A sample size of 30 participants in the REGENERATE study was deemed sufficiently statistically powered to show a signal in the primary end

point of efficacy (comparative change from baseline in BCVA in the treated and control eyes) based on power calculations from the original phase I/II study (NCT01461213).

Study setting

The REGENERATE study was conducted at two NHS eye hospitals, namely, the Oxford Eye Hospital and Moorfields Eye Hospital. These sites were selected based on their expertise and prior experience in conducting clinical trials of retinal gene therapies and their access to specialist clinics for patients with inherited retinal diseases.

Participants

Participants were males aged 18 years or above, with a clinical phenotype of choroideremia, confirmed genetic or molecular diagnosis and having BCVA better than or equal to 6/60 (20/200; LogMAR 1.0). Candidates were excluded if they had an additional cause for sight loss (e.g. amblyopia) or any other significant ocular and non-ocular disease or disorder which, in the opinion of the investigator, might put them at risk through participation in the study.

Allocation for treatment

Participants in the REGENERATE study were assigned to one or both of the following cohorts:

- Cohort 1 included all participants and compared changes in BCVA and other measures of visual function in the treated eye against baseline values.
- Cohort 2 included the subset of participants with symmetrical disease for whom selection of the treated eye was randomised and compared the rate of anatomical degeneration in the treated eye and the untreated contralateral (control) eye.

In the participants with asymmetrical disease (and therefore not included in Cohort 2), the decision about which eye to treat was made on clinical grounds and the worse-affected eye was chosen.

Note that randomisation was not used for assigning treatment (vs. placebo/standard care as in randomised controlled trials) to participants included in Cohort 2, but solely for selection of the eye to be treated in these participants for whom the progress of retinal degeneration was relatively symmetrical between the two eyes, defined as

- a difference in BCVA of no more than one line of letters, as measured on an Early Treatment Diabetic Retinopathy Study chart; and
- no more than 25% difference in the area of surviving RPE, as measured by fundus autofluorescence.

Intervention

Adeno-associated virus serotype 2 vector encoding Rab escort protein 1 vector suspension (1×10^{12} vector particles per ml) was supplied by Nightstar Therapeutics (London, UK), now part of Biogen Inc. (Cambridge, MA, USA). Up to 0.1 ml of AAV2-REP1 vector suspension, corresponding to a dose of up to 1×10^{11} vector particles, was administered to the treated eye by subretinal injection. The dose varied slightly dependent on the amount of residual retina. Hence, in more advanced patients, 0.03–0.05 ml might have been sufficient to detach the target area of retina completely. The concentration of vector remained the same.

The surgical technique for subretinal administration of the AAV2-REP1 vector suspension has been described previously (MacLaren RE, Groppe M, Barnard AR, Cottriall CL, Tolmachova T, Seymour L, *et al*. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. *Lancet* 2014;**383**:1129–37). All surgeries took place at the participating hospital sites using the standard BIOM® (binocular indirect ophthalmic microscope) operating system (OCULUS Optikgeräte GmbH, Wetzlar, Germany). The retina was detached with 0.1–0.5 ml of balanced salt solution injected through a

subretinal cannula connected to a vitreous injection set. A dose of up to 1×10^{11} vector particles was then injected into the subretinal fluid through the same entry site.

Control eyes received no intervention.

Assessments

Functional assessments included BCVA, contrast sensitivity, dark adaptometry and central visual field mapping (using microperimetry), while anatomical assessments included imaging techniques such as optical coherence tomography and fundus autofluorescence. Safety tests included assessments of ocular and general physical condition, as well as standard blood tests of common physiological markers. Biological samples were also analysed to monitor vector shedding and immunogenicity.

Cohort 1 comprised the 30 participants who enrolled in this study, of whom 28 participants had symmetrical disease and were therefore also assigned to Cohort 2. The statistical analyses were therefore performed on the total cohort comparing visual function and anatomical degeneration in treated versus control eyes. Summary statistics of each assessed variable was performed for treated eyes versus control eyes. Data involving a comparison of an assessed variable between the treated and untreated eyes (of each participant) were estimated as the difference between the eyes (with a 95% confidence interval) and simple analysis of change from baseline (paired *t*-test) was performed at 24 months post treatment. As most participants (93%) had symmetric disease, analysis of covariance was deemed unnecessary.

Results

Clinical outcomes

Overall, BCVA remained relatively stable in treated and control eyes over the 24-month assessment period.

A statistically significant deterioration in visual fields was observed in treated eyes compared with control eyes, possibly caused by surgery-induced damage in some treated eyes as manifested by increased loss of autofluorescence and retinal sensitivity at the edges of surviving islands of retinal tissue. The treated eyes underwent retinal detachment prior to injection of the vector suspension into the subretinal space, and this procedure itself can lead to some degree of reduction in central retinal sensitivity and autofluorescence. However, the statistical significance of the comparative reduction in visual fields in treated and control eyes was lost when central retinal sensitivity and area of autofluorescence were analysed in uncomplicated cases. In eyes with no complications, retinal degeneration continued centripetally in both treated and control eyes.

Safety

Most adverse events were treatable, controlled and resolved without sequelae. Six SAEs were reported in the treated eyes of four participants: one surgery-related and two inflammation-related SAEs involving clinically significant decreases in BCVA and three SAEs in one participant involving reduction in central retinal sensitivity but with BCVA remaining stable.

Discussion

Main findings of the study

There has been no signal of possible efficacy of the intervention (in terms of comparative change from baseline in BCVA between treated and control eyes) over the 24-month assessment period.

Overall, BCVA remained relatively stable in treated eyes over the 24-month assessment period. Therefore, in terms of the primary safety-related end point (change from baseline in BCVA in treated eyes at 24 months post treatment), the safety profile of the REGENERATE trial was comparable to other choroideremia gene therapy studies that evaluated the same AAV2-REP1 vector.

Limitations of the study

No evidence of possible efficacy of the intervention was observed, as a meaningful difference in comparative change from baseline in BCVA in treated and control eyes was not discernible over the 24-month assessment period. As choroideremia is a very slow degeneration, BCVA in control eyes did not decline significantly during the assessment period.

Future investigations

Future investigations will include a long-term assessment of BCVA, central retinal sensitivity and area of autofluorescence in REGENERATE trial participants in an observational study (SOLSTICE: NCT03584165). Possible approaches to further the investigation of gene therapy for choroideremia include the optimisation of inclusion criteria for subjects still retaining a healthy central retina, which may be more amenable to rescue by gene therapy.

Conclusion

Although this study has not presented evidence that reduction in visual fields caused by the intervention would be justified by the possible rescue of BCVA, a more definitive assessment may be provided by long-term monitoring of trial participants in an observational study (NCT03584165).

Trial registration

This study is registered as ISRCTN15602229 (www.isrctn.com/) and NCT02407678 (https://clinicaltrials.gov/).

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