CLINICAL TRIAL PROTOCOL

An open label Phase 2 clinical trial of retinal gene therapy for choroideremia using an adeno-associated viral vector (AAV2) encoding Rab-escort protein 1 (REP1)

Indication:	Choroideremia
Study phase:	Phase 2
Short title:	REGENERATE (REP1 Gene Replacement Therapy)
Sponsor:	University of Oxford
Ethics Reference:	15/LO/1379
EudraCT Number:	2015-001383-18
Version Number:	7.0 dated 08 July 2020
Chief Investigator:	Professor Robert MacLaren, University of Oxford
Funder:	Efficacy and Mechanism Evaluation Programme (co-funded by the Medical Research Council and the National Institute for Health Research)

Chief Investigator Signature: Robert MacLaren

Conflict of Interest Statement
The Chief Investigator of the study, Professor Robert MacLaren, is a consultant to several gene therapy companies, including Biogen Inc. which is developing the choroideremia gene therapy programme internationally. He is also employed by the University of Oxford which owns two patents relevant to choroideremia gene therapy on which he is a named inventor. He does not however receive any payments for his role in this study and does not hold shares, incentives or options in any of the companies involved.

Confidentiality Statement

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1 KEY TRIAL CONTACTS

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2 SYNOPSIS

Study title	An open label Phase 2 clinical trial of retinal gene therapy for choroideremia using an adeno-associated viral vector (AAV2) encoding Rab-escort protein 1 (REP1)
Study short title	REGENERATE (REP1 Gene Replacement Therapy)
Clinical phase	Phase 2
Study methodology	Open label, randomised, multicentre
Number of participants	30 participants in total
Study duration	24 months following administration of the investigational medicinal product, and annual review will continue thereafter as part of the participants' standard National Health Service (NHS) care
Study sponsor	University of Oxford
EudraCT number	2015-001383-18
ClinicalTrials.gov identifier	NCT02407678
Investigational medicinal product (IMP)	Adeno-associated virus serotype 2 (AAV2) encoding Rab-escort protein 1 (REP1), designated as AAV2.REP1
Dose and route of administration of IMP	Up to 10^{11} AAV2.REP1 genome particles, administered by subretinal injection
EU Orphan Drug designation of IMP	EU/3/14/1290
Non-IMP	Prednisolone, omeprazole
Study objective	The assessment of the efficacy (with respect to preservation of visual function and retinal structure) and safety of a single subretinal injection of AAV2.REP1 in participants with a confirmed diagnosis of choroideremia, as evaluated by various functional and anatomical outcomes measured over a number of time points up to 24 months post-treatment.
Primary endpoint	1. Change from baseline in best corrected visual acuity in the treated eye.
Secondary endpoints	2. Change from baseline in the central visual field in the treated eye as determined by microperimetry. 3. Change from baseline in the area of surviving retinal pigment epithelium in the treated eye as measured by fundus autofluorescence, compared to the untreated fellow eye (control eye) after randomisation of treatment to one eye or the other. 4. Change from baseline in other functional and anatomical outcomes in the treated eye pertaining to vector efficacy and safety, and safety of the subretinal injection procedure. 5. Change from baseline in immunological and physiological outcomes pertaining to vector safety.

Inclusion criteria	• Candidate is willing and able to give informed consent for participation in the study. • Male aged 18 years or above. • Genetic or molecular confirmed diagnosis of choroideremia (REP1 protein deficiency). • Active disease visible clinically within the macula region. • Best corrected visual acuity better than or equal to 6/60 (20/200; Decimal 0.1; LogMAR 1.0) in the study eye.
Exclusion criteria	• Any female, or a male aged below 18 years. • An additional cause for sight loss (e.g. amblyopia) in the eye to be treated. • Any other significant ocular and non-ocular disease or disorder which, in the opinion of the investigator, may put the participants at risk because of participation in the study. • Inability to take systemic prednisolone for a period of 45 days. • Unwillingness to use barrier contraception methods for a period of three months following gene therapy surgery. • Participation in another research study involving an investigational product in the preceding 12 weeks.

3 ABBREVIATIONS

AAV	Adeno-associated virus
AAV2	Adeno-associated virus serotype 2
AE	Adverse Event
AOSLO	Adaptive optics scanning laser ophthalmoscopy
AR	Adverse Reaction
BCVA	Best corrected visual acuity
BIOM®	Binocular indirect ophthalmic microscope
CAG	Cytomegalovirus enhancer + chicken β-Actin promoter + rabbit β-Globin splice acceptor
cDNA	Copy DNA (DNA sequence equivalent to the spliced mRNA transcript)
CHM	Choroideremia gene which provides instructions for producing Rab escort protein 1
CI	Chief Investigator
$CR_{.95}$	95% coefficient of repeatability
CRF	Case Report Form
CRO	Contract research organisation
CRP	C-reactive protein
CTIMP	Clinical trial of an investigational medicinal product
CTU	Clinical Trials Unit
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
DSUR	Development Safety Update Report
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
EME	Efficacy and Mechanism Evaluation programme
ERG	Electroretinography / Electroretinogram
ETDRS	Early Treatment Diabetic Retinopathy Study
EudraCT	European Union Drug Regulating Authorities Clinical Trials database
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GP	General Practitioner
IB	Investigator’s Brochure

ID	Identifier
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMP	Investigational medicinal product
IMPD	Investigational Medicinal Product Dossier
ISCEV	International Society for Clinical Electrophysiology of Vision
kDa	Kilodalton (unit of mass such that: 1 dalton = 1 g/mol and 1 kilodalton = 1 kg/mol)
LCA	Leber Congenital Amaurosis
logMAR	Logarithm of the Minimum Angle of Resolution
MHRA	Medicines and Healthcare Products Regulatory Agency
MIA(IMP)	Manufacturer and Import Authorisation for IMPs
MRC	Medical Research Council
mRNA	Messenger RNA
NHS	National Health Service
NETSCC	NIHR Evaluation, Trials and Studies Coordinating Centre
NIHR	National Institute for Health Research
NIMP	Non-investigational medicinal product
OCT	Optical coherence tomography
OCTRU	Oxford Clinical Trials Research Unit
OMIM	Online Mendelian Inheritance in Man database
PCR	Polymerase chain reaction
PERG	Pattern electroretinography / electroretinogram
PF-68	Pluronic F-68
PI	Principal Investigator
QP	Qualified Person
REC	Research Ethics Committee
REP1	Rab escort protein 1
RNA	Ribonucleic acid
RPE	Retinal pigment epithelium
RPE65	Gene encoding retinal pigment epithelium-specific 65 kDa protein
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard operating procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction

TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
UCL	University College London

4 INTRODUCTION

4.1 Choroideremia

Choroideremia (OMIM: 303100) is a rare, untreatable retinal degeneration that begins in childhood with loss of night vision and gradually progresses to blindness by middle age. Choroideremia is caused by loss of function of the *CHM* gene (OMIM: 300390) encoding Rab escort protein 1 (REP1) which is located on the X-chromosome (Cremers et al., 1990; Seabra et al., 1993). The disease has an X-linked recessive mode of inheritance and affects approximately 1 in 50,000 people, mostly due to loss of function (null) mutations (Sankila et al., 1992; MacDonald et al., 2009).

4.2 Rationale for Gene Therapy for Choroideremia

In vivo gene replacement therapy, where a working and healthy copy of the gene is introduced into cells within the retina, is an appealing strategy for treating choroideremia. The slow rate of degeneration means that there is a long therapeutic window in which to replace the gene in ocular cells before cell death. In addition, virtually all cases reported so far are functionally null mutations and are often predicted to result in the severe truncation or absence of endogenous REP1 protein (van den Hurk et al. 1997; McTaggart et al. 2002; van den Hurk et al. 2003; Esposito et al. 2011). This is useful because the product of the therapeutic gene will not compete with a large pool of mutant protein (MacLaren, 2009). This would limit dominant negative effects and, in theory, could mean that only a small amount of vector-delivered REP1 would be needed to arrest degeneration.

4.3 Suitability of the Eye as a Site for Gene Therapy

In its most basic concept, gene therapy is a process whereby a disease is treated at the genetic level by replacement of defective genes with functioning genes. Specially modified viruses are commonly used as vectors (i.e. carriers) in order to get copies of the functioning genes into the retinal tissue more effectively.

The eye is a very attractive site for gene therapy for several reasons –

- **Small tissue volume**: the dose of viral vector particles required to target the retina is several thousand times lower in comparison to those doses required for treatment of other organs such as the liver, which reduces the risk of immune reactions.
- **Anatomical compartmentalisation**: the target area for delivery of viral vectors is enclosed within the eye (even more so in the subretinal space) and systemic dissemination of viral vector is extremely low, which further reduces the risk of systemic immune reactions.
- **Immunological privilege**: the blood-retina barrier separates the subretinal space from the body's blood supply, and hence from most of the cell-mediated and humoral components of the immune system. Hence there is a reduced local immune response in the eye to the presence of the viral vector, which not only enhances the prospects for effective gene therapy, but also protects the eye tissue from damage caused by immune-mediated responses such as inflammation.

- **Retinal cells are post-mitotic**: the fact that most of the cells of the retina are post-mitotic (i.e. no longer actively growing and dividing) is a safety benefit because genetic modification of proliferating cells with an integrating viral vector may be associated with an increased risk of malignancy.
- **Easy accessibility**: surgical access is relatively easy and the route of administration can be altered to enable targeting of either the:
  - Inner retina (comprising retinal neurons such as bipolar cells, ganglion cells, horizontal cells and amacrine cells) by intravitreal injection.
  - Outer retina (comprising rod and cone photoreceptors) and retinal pigment epithelium (RPE) by subretinal injection.
- **Ease of functional testing**: any therapeutic effect can be easily and safely evaluated by non-invasive measures of visual function and retinal health, for example –
  - Visual function tests such as assessments of visual acuity and contrast sensitivity.
  - Visual field tests such as perimetry (peripheral vision) and microperimetry (central vision).
  - Retinal function tests such as electroretinography (ERG).
  - Retinal imaging methods such as fundus photography, fundus autofluorescence, optical coherence tomography (OCT) and adaptive optics scanning laser ophthalmoscopy (AOSLO).
- **Existence of a contralateral control**: the treated eye can be compared with the untreated fellow eye (control eye) in order to determine the effect of the treatment.

4.4 Suitability of Adeno-Associated Virus as a Vector for Gene Therapy

Adeno-associated virus (AAV) is currently the favoured vector for retinal gene therapy (McClements and MacLaren, 2013). AAV has not been reported to cause disease and poses minimal risk to humans. AAV also elicits a mild immune response in comparison with other viral vectors such as adenovirus (Willett and Bennett, 2013).

Moreover, the use of AAV vectors is not accompanied by the danger of insertional mutagenesis. One concern when using other viruses, such as retrovirus or lentivirus, is the random integration events that can disrupt gene function. While AAV is unique among viruses in that it is capable of site-specific integration within the host cell genome in the absence of any apparent deleterious effects (Dutheil et al., 2009), this capability is removed in the modified AAV vectors used for gene therapy and so the risk of insertional mutagenesis is negligible. This risk is further reduced by the fact that most of the cells of the retina are post-mitotic (i.e. no longer actively growing and dividing).

AAV is also able to infect a broad range of cell types, although the infection efficiency varies based upon serotype, which is determined by the sequence of the capsid protein. These serotypes have different cellular tropism, i.e. they preferentially target specific cell types within a given host species. Many AAV vectors target neurons effectively, but only AAV serotype 2 (AAV2) has been shown to transduce both photoreceptors and the RPE after subretinal injection in non-human primates (Bennett et al. 1999; Jacobson et al. 2006a; Stieger et al., 2006; Vandenberghe et al., 2011).

4.5 Previous Clinical Trials of Retinal Gene Therapies using AAV Vectors

The world's first AAV retinal gene therapy trial took place in 2007 at Moorfields Eye Hospital and the University College London (UCL) Institute of Ophthalmology. Patients suffering from Leber Congenital Amaurosis (LCA), a type of retinal degeneration caused by defects in the *RPE65* gene encoding retinal pigment epithelium-specific 65 kDa protein, were treated with an AAV2 vector. In this case, the original viral DNA was replaced with a healthy copy of the human *RPE65* gene. The resulting AAV2.RPE65 vector was delivered via a subretinal injection, whereby a small amount of fluid containing a high concentration of the vector genome particles was injected underneath the retina in a short surgical procedure.

The initial published results of the Moorfields clinical trial (Bainbridge et al., 2008) provided evidence that the AAV2.RPE65 vector was safe at delivering the RPE65 gene into the targeted retinal cells. The induced retinal detachment resolved spontaneously and fully within a few days after injection, with subsequent recovery of vision to pre-existing levels. No clinically significant adverse effects of subretinal vector delivery were detected, and the absence of systemic dissemination suggested that any extraocular leakage of vector from the subretinal space was minimal. These findings were supported by the preliminary results of two other trials of gene therapies for LCA also using AAV2.RPE65 vectors, reported around the same time by other research groups in the USA (Maguire et al., 2008; Hauswirth et al., 2008).

Although the treatment effects of the AAV2.RPE65 vector used in the Moorfields clinical trial did not last indefinitely (Bainbridge et al., 2015), it should be noted that the promoter (gene activator) used for the vector in this trial was derived from the human *RPE65* gene, and it is likely that the expression of cell-specific promoters will be eventually suppressed in diseased cells (MacLaren, 2009). AAV2.RPE65 vectors containing the strong CAG[1] ubiquitous promoter have provided evidence of sustained expression over several years (since 2000) without evidence of long term fade in *RPE65*-knockout dogs (Jacobson et al., 2006b; Bennicelli et al., 2008), as well as efficacious transduction in human participants in clinical trials conducted by two independent research groups in the USA (Maguire et al., 2008; Hauswirth et al., 2008). Subsequent data from both of these two independent trials (Simonelli et al. 2010; Jacobson et al., 2012; Testa et al., 2013) have provided further evidence that retinal gene therapy involving subretinal administration of the AAV2.RPE65 vector is safe and that the therapeutic effect is maintained up to 3 years after receipt of gene therapy; although it must be noted that visual function has not been preserved in a subset of participants (Cideciyan et al., 2013; Jacobson et al., 2015) indicating that other factors, such as the method of administration of the vector, are also involved in the success of retinal gene therapy.

[1] The CAG synthetic promoter (Niwa et al., 1991) is a strong ubiquitous promoter which is constructed from the following sequences:

- C = Cytomegalovirus early enhancer element
- A = the promoter, the first exon and the first intron of chicken beta-Actin gene
- G = the splice acceptor of the rabbit beta-Globin gene

4.6 Clinical Trials of a Retinal Gene Therapy for Choroideremia

In 2011, a Phase 1/2 clinical trial (ClinicalTrials.gov: NCT01461213; EudraCT: 2009-014617-27) of a gene therapy for choroideremia was commenced, using a modified AAV2 vector having a CAG promoter and the REP1 cDNA sequence (i.e. the exonic regions of the *CHM* gene encoding REP1), designated as AAV2.REP1. Further to the positive initial results from the first 6 choroideremia patients treated with the AAV2.REP1 vector (MacLaren et al., 2014), followed by publication of data showing that the effect of gene therapy was sustained indefinitely (Edwards et al., 2016), regulatory approval of this Phase 2 clinical trial (ClinicalTrials.gov: NCT02407678; EudraCT: 2015-001383-18) was obtained to test the AAV2.REP1 vector in an additional 30 choroideremia patients.

Subsequently, the 24 month results of all 14 choroideremia patients treated the original Phase 1/2 clinical trial have been published (Xue et al, 2018). The median best corrected visual acuity[2] (BCVA), measured using Early Treatment Diabetic Retinopathy Study (ETDRS) charts, improved by 4.5 letters across all 14 treated eyes, while declining by −1.5 letters across all 14 untreated eyes. The trial thus met its primary endpoint of improving vision following gene therapy compared to untreated fellow eyes, despite any potential adverse effects of retinal detachment. In 12 out of 14 patients, the retinal gene therapy led to recovery of visual acuity in all eyes and variable degrees of acuity gains that generally occurred within 6 months of treatment and were sustained up to 5 years.

Results published from three parallel Phase 2 clinical trials testing the same batch of AAV2.REP1 vector have all shown good safety results from subretinal administration, and gains of more than 3 lines of vision (measured with ETDRS charts) in 2 of the 18 patients enrolled (Dimopoulos et al; 2018; Fischer et al., 2019; Lam et al., 2019).

[2] Best corrected visual acuity = visual acuity measured after refractive errors in the eye, such as myopia, hypertropia, astigmatism and presbyopia, have been corrected with eyeglasses or contact lenses where necessary.

5 STUDY OBJECTIVE AND ENDPOINTS

5.1 Study Objective

The assessment of the efficacy (with respect to preservation of visual function and retinal structure) and safety of a single subretinal injection of AAV2.REP1 in participants with a confirmed diagnosis of choroideremia, as evaluated by various functional and anatomical outcomes measured over a number of time points up to 24 months post-treatment.

5.2 Primary Endpoint

- Change from baseline in BCVA in the treated eye.

5.3 Secondary Endpoints

- Change from baseline in the central visual field in the treated eye as determined by microperimetry.
- Change from baseline in the area of surviving RPE in the treated eye as measured by fundus autofluorescence, compared to the untreated fellow eye (control eye) after randomisation of treatment to one eye or the other.
- Change from baseline in other functional and anatomical outcomes in the treated eye pertaining to vector efficacy and safety, and safety of the subretinal injection procedure.
- Change from baseline in immunological and physiological outcomes pertaining to vector safety.

6 PARTICIPANT RECRUITMENT

6.1 Eligibility for Participation in the Study

Participants will comprise males with a clinical phenotype of choroideremia and a confirmed diagnosis. Male participants are chosen because choroideremia is an X-linked recessive disorder which affects primarily males. Females are carriers of the disease. Some females may manifest a clinical phenotype similar to that seen in affected males; however, in this study only males will be treated.

6.2 Confirmation of Diagnosis of Choroideremia

Confirmation of a diagnosis of choroideremia precedes the invitation of a candidate to participation in the study and to the initial screening visit. Confirmation of diagnosis involves, but is not limited to, the following assessments (for both eyes):

- Provision of demographic, medical and ocular history.
- Full ophthalmic examination.
- Area of surviving RPE measured by fundus autofluorescence.
- Genetically confirmed diagnosis of choroideremia. Participants without a confirmed mutation in the REP1 cDNA (i.e. the exonic regions of the *CHM* gene that encode REP1), but who have the clinical phenotype typical of choroideremia confirmed by a minimum of three independent retinal specialists can only be included in the study if they meet all the following three criteria:
    i. **Family history consistent with X-linked inheritance**. Since choroideremia has a distinct clinical appearance compared with other types of retinal degeneration and an X-linked inheritance, the appropriate family history will provide strong evidence that the affected persons have choroideremia even though a mutation in the *CHM* gene cannot be detected. (Note that the REP1 deficiency still needs to be verified as detailed in the following criterion.)
    ii. **Absent REP1 on a western blot of a blood sample**. Despite being unable to find a mutation in (the exons of) the *CHM* gene, an absence of REP1 in these affected persons may be caused by other factors such as a mutation within an intron affecting splicing of mRNA expressed by the *CHM* gene, or a mutation in the *CHM* promoter region. Since the REP1 protein is expressed ubiquitously, deficiency of the *CHM* gene product can be confirmed by screening peripheral blood for REP1 by a western blot, and REP1 function can be assessed through a prenylation assay (MacDonald et al., 1998). The absence of REP1 on a western blot provides a molecular confirmation of the clinical diagnosis of choroideremia.
    iii. **Normal *RPE65* gene on sequencing**. The *RPE65* gene codes for retinal pigment epithelium-specific 65 kDa protein. Mutations in *RPE65* have been associated with LCA type 2 and retinitis pigmentosa. Early in the course of the disease, choroideremia may share similar features with dominant *RPE65* disease, since both have symptoms of night blindness and circumferential loss of RPE. However, differences emerge in time as the disease progresses.

6.3 Inclusion Criteria

Inclusion criteria for participation in the study comprise:

- Candidate is willing and able to give informed consent for participation in the study.
- Male aged 18 years or above.
- Genetic or molecular confirmed diagnosis of choroideremia as detailed in the preceding section.
- Active disease visible clinically within the macula region.
- BCVA better than or equal to 6/60 (20/200; Decimal 0.1; LogMAR 1.0) in the study eye.

6.4 Exclusion Criteria

Exclusion criteria for participation in the study comprise:

- Any female, or a male aged below 18 years.
- An additional cause for sight loss (e.g. amblyopia) in the eye to be treated.
- Any other significant ocular and non-ocular disease or disorder which, in the opinion of the investigator, may put the participants at risk because of participation in the study.
- Inability to take systemic prednisolone for a period of 45 days.
- Unwillingness to use barrier contraception methods for a period of three months following gene therapy surgery.
- Participation in another research study involving an investigational product in the preceding 12 weeks.

6.5 Recruitment

A database will be compiled of choroideremia sufferers who have expressed an interest in participating in the Phase 2 study. The Chief Investigator (CI), who has access to all the medical records, will shortlist candidates who meet the preceding inclusion criteria and who might be expected to benefit from gene therapy. An invitation to participate in the study will be extended to shortlisted candidates. Shortlisted candidates will be sent printed copies of the latest approved versions of the Informed Consent Form (ICF) and the Participant Information Sheet (PIS). The PIS will provide information on the following topics, among others:

- The exact nature of the study.
- What participation in the study will involve.
- The possible benefits and risks of participation in the study.

Shortlisted candidates who are interested in participating in the study may contact the CI via the contact details provided in the PIS. Interested candidates will be invited to meet with an investigator in order to give their informed consent to participate in the study, as detailed in the following section.

6.6 Informed Consent

Interested candidates will be invited to meet with an investigator in order to give their informed consent to participate in the study. The following specific issues will be discussed by the investigator in addition to any matters of particular interest or concern to the prospective participant:

- The potential for no benefit.
- The potential for loss of vision due to complications of surgery, such as infection, retinal detachment, haemorrhage and cataract.
- The potential for loss of vision due to eye inflammation caused by immune reactions.
- The theoretical potential for malignancy.
- The possibility of spread of the vector to other organs and germline transmission.
- The nature and duration of follow-up tests required for the study.

The decision about which eye to treat will be made on clinical grounds and will generally be the worse eye affected in cases where BCVA differs between the two eyes by 2 lines or more of ETDRS letters. The eye to be treated will be randomised in cases where the degeneration is relatively symmetrical between the two eyes, defined as:

- a difference in BCVA of no more than 1 line of ETDRS letters, and
- no more than 25% difference in the area of surviving RPE as measured by fundus autofluorescence.

The following treatment options will be discussed in detail and agreed with each prospective participant as part of the informed consent process:

- Prospective participants having non-symmetrical retinal degeneration will be allocated to the non-randomised arm. The treated eye will generally be the worse eye.
- Prospective participants having relatively symmetrical retinal degeneration will be allocated to the randomised arm. It will be made clear in discussion with the prospective participants that they will not know which eye will be selected for treatment ahead of surgery. This requirement is necessary in order to avoid the possibility of selection bias.

Prospective participants will be allowed as much time as wished to consider the information provided and, if necessary, to consult with their general practitioner (GP) or other independent parties so as to make an informed decision about their participation in the study. Another meeting with the investigator will be scheduled if more time for deliberation is required by the prospective participant.

Written informed consent is considered given once the latest approved version of the ICF has been signed and dated by the prospective participant and by the investigator who presented and obtained the written informed consent. The investigator who obtains the consent must be suitably qualified and experienced, and have been authorised to do so by the Principal Investigator (PI) of the relevant site. Before the ICF is signed by both parties, the investigator is obliged to explain clearly that the prospective participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal. A copy of the signed ICF will be given to the participant. The original signed ICF will be retained in the Investigator Site File (ISF) and a copy kept in the medical notes.

7 STUDY OVERVIEW

7.1 Description of the Study

This is an open label study, since both the investigator and the participant are unmasked to the study procedure (i.e. subretinal injection of the AAV2.REP1 vector). The study procedure involves a surgical intervention and therefore it is not ethically viable to have a masked surgical procedure performed.

The selection of the eye to be treated will be randomised in participants who have symmetrical disease (as discussed in the previous section). The untreated fellow eye of randomised participants will be used as an internal control.

Participants who are clearly dependent on one eye will have treatment of the worse eye, as long as the inclusion criteria for this study are met. In this case, the decision about which eye to treat will be made on clinical grounds, and will be discussed in detail and agreed with each participant prior to enrolment in the study as part of the informed consent process.

7.2 Design of the Study

The entry criteria of this Phase 2 study have been widened to include participants in whom the fellow eye cannot be used as a control. This is because improvements in maximal sensitivity (the dimmest stimulus that can be seen) after surgery were noted in the treated eyes of participants in the Phase 1 study. Hence visual function to the pre-operative level can be compared in the same eye. This also permits the inclusion of participants in the terminal stages of sight loss who would otherwise be completely blind by the time this gene therapy becomes an approved treatment.

For assessment of the anatomical rate of degeneration, however, the fellow untreated eye would ideally be required as an internal control which, in turn, requires the inclusion of participants having a fairly symmetrical disease. Treatment of one eye or the other would also need to be randomised in order to avoid selection bias.

Hence we shall have two cohorts of participants:

- Cohort 1 will include all participants and will make comparisons of visual function in the treated eye before and after surgery in relation to vector dose per unit area of retina.
- Cohort 2 will be the subset of participants in whom treatment has been randomised and will make comparisons to the fellow untreated (control) eye with regard to the rate of anatomical degeneration.

7.3 Occurrence of Adverse Events during the Study

Any Adverse Event, irrespective of its perceived relationship to the AAV2.REP1 vector and/or the surgical procedure, will be captured in the participant's medical records. The relevant data will be recorded in the appropriate paper Case Report Form (CRF) and entered into the trial database following the general procedure for recording of trial data described in Section 13.3. The relevant safety procedures will be followed as detailed in Section 10.

7.4 Plan of the Study

7.4.1 Schedule of Study Visits

A complete list of the study assessments is provided in Table 1 in Section 8.1. A comprehensive schedule of the visits and visit-specific assessments for the 24 month duration of the study is provided in Table 2.

7.4.2 Visit 1 (Screening)

At the initial screening visit the participants will be taken through a number of assessments (as detailed in Table 2) designed to determine their medical condition, and to obtain baseline values against which vector safety and efficacy will be evaluated. The participants' demographic, medical and ocular history will be reviewed, and concomitant medications will be recorded.

If written informed consent has not yet been obtained from the said participants, the ICF will also be signed and dated at this visit by the participants and by the investigators who presented and obtained the written informed consent.

The screening visit (Visit 1) should be scheduled within 8 weeks of the anticipated date of surgery (Visit 2).

Prednisolone

In order to minimise postoperative inflammation, participants will be given a 45 day course of oral prednisolone, starting at a daily dose of 1 mg per kg of body weight (rounded to the nearest multiple of 5 mg) and gradually tapering off to 10 mg and 5 mg in the second last and last weeks:

Days	**Daily dose/kg**	**Daily dose (rounded to nearest multiple of 5 mg) for adult weighing:**				
		60 kg	**70 kg**	**80 kg**	**90 kg**	**100 kg**
3 days before surgery	1 mg/kg	60 mg	70 mg	80 mg	90 mg	100 mg
7 days after surgery	1 mg/kg	60 mg	70 mg	80 mg	90 mg	100 mg
7 days	0.75 mg/kg	45 mg	55 mg	60 mg	70 mg	75 mg
7 days	0.5 mg/kg	30 mg	35 mg	40 mg	45 mg	50 mg
7 days	0.25 mg/kg	15 mg	20 mg	20 mg	25 mg	25 mg
7 days	0.1 mg/kg	10 mg	10 mg	10 mg	10 mg	10 mg
7 days	0.05 mg/kg	5 mg	5 mg	5 mg	5 mg	5 mg

Prednisolone tablets will be taken once a day, preferably in the morning with food. If the day of surgery is regarded as Day 0, then the 45 day course starts on Day −2 and ends on Day 42.

Omeprazole

In order to prevent gastritis, oral omeprazole (20 mg twice per day) will be given concurrently with the prednisolone (45 days in total). Omeprazole tablets may be taken with food or on an empty stomach.

7.4.3 Visit 2 (Surgery, Day 0)

The date of surgery (Visit 2) should be scheduled within 8 weeks of the screening visit (Visit 1).

Participants will be questioned for the occurrence of Adverse Events.

Surgery

The surgical procedure is conducted preferably under general anaesthesia in order to minimise the likelihood of head movements during surgery. However, it is possible for the surgical procedure to be conducted under local anaesthesia if this is judged more suitable for the participant in question.

The surgical procedure will include a standard vitrectomy, retinal detachment and administration of a subretinal injection of AAV2.REP1 (up to 1×10^{11} genome particles) in the treated eye, as explained in greater detail in Section 9.4.

Participants will remain overnight at the relevant hospital site, and will be carefully monitored for the occurrence of Adverse Events peri- and post-operatively.

Prednisolone

Since no food can be taken on the day of surgery, the oral prednisolone is given with food immediately after recovery from theatre to avoid administration on an empty stomach.

7.4.4 Visits 3-11 (Follow-Up, Day 1 to Month 24)

Participants will be required to attend a further 9 follow-up visits (Visits 3-11) over a 24 month period for a number of assessments designed to determine their medical condition, and to evaluate vector safety and efficacy.

Participants will be initially followed up 1 day, 7 days and 1 month following surgery (Visits 3-5). Visit 3 is on Day 1 post-surgery; therefore the participants will already be present following their overnight stay at the relevant hospital site after surgery on Day 0.

Thereafter, follow-up visits will continue every 3 months up to 12 months post-surgery (Visits 6-9) and then every 6 months until 24 months post-surgery (Visits 10-11).

Details of the specific assessments to be conducted at Visits 3-11 are provided in Table 2.

7.4.5 Unscheduled Visit

At the investigator’s discretion, a participant may attend for an unscheduled study visit. Details of the specific assessments to be conducted at an unscheduled visit are provided in Table 2.

7.4.6 Early Termination Visit

A participant may withdraw from the study at any time (see Section 7.4). If a participant is withdrawn, then every reasonable effort is to be made to complete the schedule of assessments described for the Early Termination Visit in Table 2. A reason for the participant's withdrawal, if available, will be documented in the participant's medical notes and in the CRF.

7.4.7 Concomitant Medication

Details of concomitant medication will be collected at the screening visit, and updated at every study visit (including any unscheduled visits). Throughout the study, investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care. Any medication (including anaesthetic and other surgical medications, but excluding study medication) taken during the study will be recorded in the participant's medical notes and reported in the CRF.

7.5 Discontinuation/Withdrawal from the Study

Each participant has the right to withdraw from the study at any time. In addition, the PI of the relevant site may discontinue a participant from the study at any time if the PI considers it necessary for any reason including:

- Significant protocol deviation.
- Significant non-compliance with study requirements.
- An Adverse Event which results in inability to continue to comply with study assessments.
- Withdrawal of consent.
- Lost to follow-up.

Annual checks of general health will however need to be monitored continuously and every effort will be made to ensure that participants understand the need for this monitoring at consent. Withdrawal from the study will not necessarily result in exclusion of the data acquired up to the point of withdrawal. The reason for withdrawal will be recorded in the participant's medical records and in the CRF. If the participant is withdrawn due to an Adverse Event, the PI of the relevant site will arrange for follow-up visits until the Adverse Event has resolved or stabilised.

7.6 End of the Study

The study ends once the 24 month post-surgical assessments have been completed for all participants.

7.7 The Possibility of Post-Study Treatment

The aim of this Phase 2 study is to evaluate AAV2.REP1 gene therapy for choroideremia, with the ultimate goal of developing a safe and efficacious licensed medicinal product for the treatment of this currently incurable retinal disease. Naturally, any approved gene therapy for a retinal disease should ideally be able to treat both eyes of sufferers. An investigation undertaken during the University of Pennsylvania LCA gene therapy study has demonstrated that re-administration of the AAV2.RPE65 vector in the second eye of participants can be performed safely and without loss of vector efficacy (Bennett et al., 2012). Therefore, in the future there is every possibility that participants in this study may be eligible to be considered for gene therapy for their second eyes. Part of the reason for limiting any immune response by the concomitant use of oral prednisolone is to keep open the option of administering a second treatment to participants at a later date. This decision will depend on a number of factors including the condition of their second eye and the availability of the intervention.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 Study Assessments

A detailed description of the purpose and the manner of conducting each of the following study assessments listed in Table 1 below will be set out in the relevant study-specific standard operating procedure (SOP). Copies of the relevant SOPs will be retained in the Trial Master File (TMF) and ISFs.

Table 1: Assessments of functional outcomes, anatomical outcomes and safety

Assessments of functional outcomes	**Purpose of assessments**
BCVA test (with compensation for refractive error)	Assessment of visual acuity
Contrast sensitivity test (with compensation for refractive error)	Assessment of visual contrast sensitivity
Microperimetry	Measurement of the central visual field
Perimetry	Measurement of the peripheral visual field
Dark adaption and full-field stimulus threshold tests (night vision tests)	Assessment of rod photoreceptor function
Colour vision tests	Assessment of cone photoreceptor function
Pattern ERG (PERG)	Assessment of retinal function (i.e. the electrical responsiveness of the retina)
Assessments of anatomical outcomes	**Purpose of assessments**
Fundus autofluorescence imaging	Measurement of the area of surviving RPE
OCT imaging	Measurement of retinal thickness and assessment of retinal and choroidal structure
AOSLO imaging	Enumeration of cone photoreceptors and assessment of the cone photoreceptor mosaic

Safety assessments	Purpose of assessments
Full ophthalmic examination (including dilated ophthalmoscopy, slit lamp examination and assessment of intraocular pressure and lens opacity)	Assessment of ocular condition
Fundus photography	Assessment of ocular condition
Fluorescein angiography	Assessment of ocular condition
Indocyanine green angiography	Assessment of ocular condition
Vital signs (resting pulse and blood pressure)	Assessment of general physical condition
Blood samples taken for standard blood chemistry assays:	
• Glucose test	Assessment of general physical condition
• Routine kidney function tests, e.g. for creatinine, urea and electrolytes	Assessment of general physical condition
• Routine liver function tests, e.g. for total protein, albumin, aspartate transaminase / alanine transaminase ratio, alkaline phosphatase and bilirubin	Assessment of general physical condition
• Full blood counts	Assessment of general physical condition
Blood samples taken for immunological assays:	
• Immunochemistry assays, i.e. assays for C-reactive protein (CRP), total immunoglobulin M (IgM) and total immunoglobulin G (IgG)	Assessment of AAV2.REP1 immunogenicity
• Immunoassays, i.e. enzyme-linked immuno-sorbent assay (ELISA) and enzyme-linked immunospot (ELISPOT)	Assessment of AAV2.REP1 immunogenicity
Samples of blood, saliva, tears and urine taken for polymerase chain reaction (PCR) assays for vector shedding.	Assessment of AAV2.REP1 dissemination

8.2 Masking of Study Assessments

This is an open label study with no masking. However, in order to minimise bias evaluation of the treated eye and untreated fellow eye (control eye), the following ophthalmic assessments will be conducted by an appropriately qualified masked observer once the participant's treated eye has had time to heal after the surgical procedure and has regained its normal appearance and function:

- BCVA test (preceded by refractive error measurement)
- Contrast sensitivity test (preceded by refractive error measurement)
- Microperimetry
- Perimetry
- Dark adaptation and full-field stimulus threshold tests
- Colour vision tests
- PERG
- Fundus autofluorescence imaging
- OCT imaging
- AOSLO imaging
- Full ophthalmic examination
- Fundus photography
- Fluorescein angiography
- Indocyanine green angiography

Participants will be advised by the investigator not to disclose to the masked observer which eye has been treated.

8.3 Anonymisation of Data from Study Assessments

Data from study assessments will be recorded in the CRF in an anonymised manner, i.e. data will be recorded in association with a study identifier (ID) particular to each participant. On no occasion will data be recorded in the CRF in association with the name and any other identifying details of a participant.

8.4 Study Assessments Requiring Biological Samples

8.4.1 Collection and Processing of Biological Samples

The following biological samples will be collected by a member of the study team from the participant at the relevant visits specified in Table 2:

- Blood samples (up to 60 ml in total) will be drawn in accordance with local National Health Service (NHS) hospital site procedures and guidelines and processed in accordance with the relevant study-specific SOPs.
- A saliva sample (up to 3 ml) will be taken and processed in accordance with the relevant study-specific SOP.
- Tear swabs (both eyes) will be taken and processed in accordance with the relevant study-specific SOP.

- A urine sample (up to 10 ml) will be taken and processed in accordance with the relevant study-specific SOP.

Note that a duplicate set of sample vials will be prepared for each assessment – one set to be sent for analysis, and another set to be stored locally by the clinical research team at −60°C or lower until the end of the study, in order to allow for re-testing of anomalous results. During local storage, physical access to the stored biological samples will be restricted to the clinical research team only.

8.4.2 Analysis of Biological Samples

Analysis of the biological samples (i.e. blood, saliva, tears and urine) taken from participants will be undertaken by local NHS hospital laboratories or by a contract research organisation (CRO):

- Biological samples for analysis by local NHS hospital laboratories –
  - Blood for standard blood chemistry assays (glucose test, routine kidney function tests, routine liver function tests, full blood counts).
  - Blood for immunochemistry assays (CRP, IgM and IgG).

  The biological samples in question will be sent immediately to the local NHS hospital laboratory for analysis in accordance with local NHS hospital site procedures and guidelines. Note that these samples and data from the analysis thereof are not anonymised. Test results from the local NHS hospital laboratories will be returned to the study team for inclusion in an anonymised manner in the CRF.
- Biological samples for analysis by a CRO –
  - Blood for immunoassays (ELISA and ELISPOT).
  - Blood, saliva, tears and urine for PCR assays for vector shedding.

  The biological samples in question will be anonymised by labelling with the appropriate study ID specific for each participant, and will be temporarily stored locally by the clinical research team at −60°C or lower, prior to despatch to a CRO for analysis. During local storage, physical access to the stored samples will be restricted to the clinical research team only. Test results from the CRO will be returned to the study team for inclusion in an anonymised manner in the CRF.

8.4.3 Retention of Biological Samples

Biological samples (i.e. blood, saliva, tears and urine) taken from participants will be retained for the following periods:

- Biological samples sent for analysis to local NHS hospital laboratories will not be stored long-term, but will be retained by the relevant laboratories for a that period of time specified by local National Health Service (NHS) hospital site procedures and guidelines.
- Biological samples sent for analysis to a CRO will be retained until the end of the study.
- Duplicate sets of biological samples stored locally by the clinical research team at −60°C or lower will be retained until the end of the study.

Biological samples obtained from participants will generally not be stored beyond the duration of this study, nor will these biological samples be used in other studies or for other purposes.

8.5 Location of Study Assessments

All study assessments and the study procedure (i.e. subretinal injection of the AAV2.REP1 vector) will take place in the participating hospital sites, and participants will be reimbursed for their travel-related expenses (see Section 16.6).

8.6 Schedule of Study Assessments

A schedule of the visits and visit-specific assessments for the 24 month duration of the study is provided in Table 2. All the visit-specific assessments listed in Table 2 are compulsory, with the exception of the AOSLO assessment at 24 months which is optional depending on the outcome of the baseline assessment. That is to say, if no reliable cone imaging can be obtained by an experienced operator at screening then the AOSLO test would not need to be repeated at a later date.

8.7 Post-Study Assessments

Participants will revert to standard NHS care at the end of the study, and will be followed-up annually as part of their standard NHS care.

Table 2: Schedule of visits and visit-specific assessments

Assessments and Procedures	Visit 1 Screening[a]	Visit 2 Day 0	Visit 3 Day 1	Visit 4 Day 7 ± 3d	Visit 5 Month 1 ± 7d	Visit 6 Month 3 ± 7d	Visit 7 Month 6 ± 21d	Visit 8 Month 9 ± 21d	Visit 9 Month 12 ± 21d	Visit 10 Month 18 ± 28d	Visit 11 Month 24 ± 28d	Early Termination Visit[b]	Unscheduled Visit[c]
Informed consent	X												
Demographic, medical and ocular history	X												
Full ophthalmic examination[d]	X		X	X	X	X	X	X	X	X	X	X	X
Prednisolone/omeprazole administration[e]	Administered daily from Day −2 to Day 42												
Randomisation (if relevant[f])		X											
AAV2.REP1 administration[g]		X											
Vital signs[h]	X		X		X							X	
Blood chemistry[i]	X				X							X	
Viral shedding[j]	X		X	X	X							X	
Immunology[k]	X				X		X					X	
Contrast sensitivity	X						X		X		X	X	
Colour vision	X						X		X		X	X	
Fundus photography	X						X		X		X	X	
Refractive error + BCVA	X			X	X	X	X	X	X	X	X	X	X
OCT + fundus autofluorescence	X		X	X	X	X	X	X	X	X	X	X	X
Microperimetry[l]	X		X	X	X	X	X	X	X	X	X	X	X
Perimetry[m]	X										X	X	
Angiography[n]	X										X	X	
AOSLO[o]	X										X		
Dark adaptation curve + full-field stimulus threshold + PERG	X								X				
Adverse Event monitoring	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X

All ophthalmic assessments (except for the surgical procedure involving subretinal injection of the AAV2.REP1 vector) are performed in both eyes.

a Screening visit (Visit 1) must be performed within 8 weeks of treatment with the AAV2.REP1 vector (Visit 2).

b Early termination visit to be performed if a participant withdraws from the study after treatment with the AAV2.REP1 vector.

[c] Full ophthalmic examination and BCVA assessment should be conducted as a minimum. Microperimetry, fundus autofluorescence and OCT assessments, as well as monitoring of concomitant medication and adverse events, may also be performed if clinically required.

[d] Full ophthalmic examination to include dilated ophthalmoscopy, slit lamp examination and assessment of intraocular pressure and lens opacity as detailed in the relevant study-specific SOP.

[e] Prednisolone is an anti-inflammatory corticosteroid. Oral prednisolone taken once a day for 45 days, commencing 2 days before treatment with the AAV2.REP1 vector (i.e. from Day −2 to Day 42). Omeprazole is an antacid, given as two doses of 20 mg per day concurrently with the prednisolone.

[f] Criteria for randomisation are outlined in Section 6.6.

[g] AAV2.REP1 vector is administered by subretinal injection to the treated eye.

[h] Pulse and blood pressure.

[i] Standard NHS blood chemistry assays comprise glucose test, routine kidney function tests, routine liver function tests, full blood counts.

[j] PCR assays are performed on blood, saliva, tears and urine samples to test for vector shedding.

[k] CRP, total IgM and total IgG immunochemistry assays and ELISA and ELISPOT immunoassays are performed on blood samples to test for vector immunogenicity.

[l] Microperimetry is used to measure the central visual field.

[m] Perimetry is used to measure the peripheral visual field.

[n] Comprises fluorescein angiography and indocyanine green angiography.

[o] AOSLO assessment at 24 months is optional depending on the outcome of the baseline assessment.

9 INVESTIGATIONAL MEDICINAL PRODUCT

9.1 Description of the Investigational Medicinal Product

The investigational medicinal product (IMP) comprises a modified AAV2 vector having a CAG promoter and the REP1 cDNA sequence (i.e. the exonic regions of the *CHM* gene that encode REP1), designated as AAV2.REP1. The AAV2.REP1 vector is owned by the Biogen Inc. and is produced to Good Manufacturing Practice (GMP) standards by the Viral Vector Core and Clinical Manufacturing Facility at Nationwide Children's Hospital in Columbus, Ohio, USA. The vector will be imported under the appropriate MIA (IMP) (Manufacturer and Import Authorisation for IMPs) license with Qualified Person (QP) release for use in the study.

The Sponsor (University of Oxford) owns the patent of the IMP (AAV2.REP1) to be used in this Phase 2 study –

- Patent title: AAV-Vectors for Use in Gene Therapy of Choroideremia
- British patent application number: 1103062.4
- First Filing (Priority) Date: 22 February 2011
- International Publication Number: WO 2012/114090 A1
- International Publication Date: 30 August 2012
- Inventors: Robert MacLaren, Miguel Seabra, Matthew During
- Applicant: Isis Innovation (on behalf of the University of Oxford)

On 4 July 2014, orphan drug designation (EU/3/14/1290) was granted by the European Commission for the AAV2.REP1 vector.

Preclinical and clinical data concerning the AAV2.REP1 vector is provided in the Investigator's Brochure (IB) prepared by Nightstar Therapeutics, now part of Biogen Inc. Information pertaining to the quality, manufacture and control of the AAV2.REP1 vector is provided in the Investigational Medicinal Product Dossier (IMPD).

9.2 IMP Dose

The AAV2.REP1 vector suspension used for gene therapy has a concentration of 1×10^{12} genome particles per ml. Up to 0.1 ml of the AAV2.REP1 suspension may be administered by subretinal injection, depending on the residual area of surviving retina present. Therefore the dose of IMP used for gene therapy may contain up to 1×10^{11} genome particles.

Calculation of vector dose is estimated based on previous studies using the AAV2.RPE65 vector (Maguire et al., 2008; Bainbridge et al., 2008; Hauswirth et al., 2008) in which maximum AAV doses (measured in viral genome particles) of 1.5×10^{10}, 1.5×10^{11} and 6×10^{10} were injected respectively. None of the doses showed any detrimental effects, but the study by Maguire et al. (2008) used Pluronic F-68 (PF-68) surfactant which prevented the adherence of AAV2 to the plastic in the injection system. In the presence of surfactant (0.001% PF-68) virtually 100% of all vector entering the injection system passed through, but without surfactant 75% of vector was lost in the injection system, presumably as a result of internal binding of AAV2 to the plastic (Bennicelli et al., 2008). Data on final titre without surfactant in the other two studies is not available, but it seems reasonable to assume that the viral dose actually injected would have been less than the pre-injection titre. The second phase of the dose escalation study led by Maguire was completed without adverse reactions in three participants receiving a dose of 1.5 x 10^{11} (Maguire et al., 2009). Hence this study (Maguire et al., 2008; Maguire et al., 2009) probably has used the highest dose of AAV genomes definitely known to have been injected and shown to have been well tolerated.

An initial dose of up to 1 x 10^{10} genome particles (with the addition of surfactant) was used for the first 6 participants in the original Phase 1/2 study of a gene therapy for choroideremia (MacLaren et al., 2014; Edwards et al., 2016; Xue et al., 2018). The dose was well tolerated. In addition, another 5 participants have now been dosed with up to 1 x 10^{11} genome particles (with the addition of surfactant) with no significant safety issues reported (see IB). A dose of up to 1 x 10^{11} genome particles will be used in this Phase 2 study, as the dose used to date is within the range where good safety data in humans is available.

9.3 Storage of IMP

The IMP (AAV2.REP1 vector suspension) is stored at −60°C or lower prior to use. Shortly before surgery, a sufficient number of vials of the AAV2.REP1 vector suspension (corresponding to a total volume of no less than 0.2 ml) are withdrawn from storage and transported to the operating theatre, in accordance with local hospital policies and procedures in force at each site. The AAV2.REP1 vector suspension is permitted to thaw en route to the operating theatre. Should surgery be delayed for whatever reason, the AAV2.REP1 vector suspension may be stored temporarily at 4°C in a refrigerator in the vicinity of the operating theatre until required. Stability tests have shown that the vector can be kept at 4°C for at least one week.

9.4 Administration of IMP

The vial label is checked, and the IMP is then checked for clarity. The surgeon is responsible for checking that the correct aliquot of IMP is visible in each vial and that all labels are correct before commencing the operation. The vector is subsequently drawn up into the injection syringe under sterile conditions as documented in the relevant study-specific SOP.

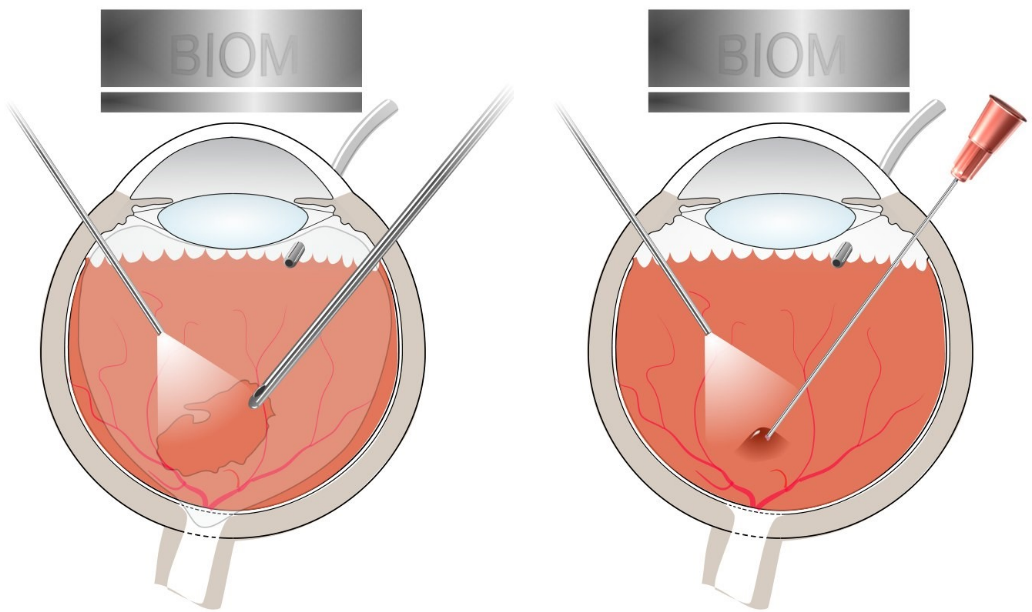
All surgery will take place in the participating hospital sites using the standard BIOM® (binocular indirect ophthalmic microscope) operating system (Spitznas, 1987). A small gauge sutured approach will be used to avoid any potential risks of wound leakage. Participants will undergo a standard vitrectomy and detachment of the posterior hyaloid (Figure 1). The retina will be 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 10^{11} genome particles vector suspension will then be injected into the subretinal fluid through the same entry site.

The subretinal injection will target any area of the macula but also include the fovea if possible. In each case the vector will be injected so that the subretinal fluid overlies all edge boundaries of the central region that has yet to undergo chorioretinal degeneration, as identified by fundus autofluorescence imaging. Targeting of AAV2.REP1 to the most dynamic leading edge of the degeneration will maximise the chance of identifying a treatment effect within the two year study timeframe.

Additional detail is provided in the relevant study-specific SOP.

Figure 1: Subretinal injection of AAV2 vector

A B



(A) A standard vitrectomy through the BIOM® operating system to remove the vitreous gel, is followed by (B) injection of vector suspension (up to 0.1 ml) through a cannula into the subretinal space.

10 SAFETY REPORTING

10.1 Definitions

Definitions of the various categories of Adverse Events are set out in Table 3 below.

Table 3: Categories of Adverse Events

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an IMP which is related to any dose administered to that participant. All cases judged by the reporting investigator as having a reasonable suspected causal relationship to the IMP, i.e. the relationship cannot be ruled out, qualify as Adverse Reactions.
Serious Adverse Event (SAE)	Any untoward medical occurrence that: • results in death, • is life-threatening, • requires inpatient hospitalisation or prolongation of existing hospitalisation, • results in persistent or significant disability or incapacity, • consists of a congenital anomaly or birth defect, or • is an important medical event that may not be immediately life-threatening, incapacitating or requiring hospitalisation, but which jeopardises the participant or requires an intervention to prevent one of the other outcomes listed above.
Serious Adverse Reaction (SAR)	A Serious Adverse Event that is, in the opinion of the reporting investigator, believed with reasonable probability to be related to the IMP based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A Serious Adverse Reaction, the nature and severity of which is not consistent with the safety data of the IMP in question as described in the IB.

Please note that, in order to avoid confusion or misunderstanding of the difference between the terms 'serious' and 'severe', the following clarification is provided: 'severe' is often used to describe the intensity of an AE, which may be of relatively minor medical significance, while 'serious' has the regulatory definition supplied above.

10.2 Causality

The relationship of each AE to the IMP must be determined by the reporting investigator according to the following definitions:

Related: The AE follows a reasonable temporal sequence from administration of the IMP. It cannot reasonably be attributed to any other cause.

Not Related: The AE is probably produced by the participant's clinical state or by other modes of therapy administered to the participant.

10.3 Expectedness

Expectedness of AEs will be determined according to the summary of safety data provided in the IB. The clinical and non-clinical safety information in the IB has been compiled from the data collected during the preceding Phase 1/2 study (MacLaren et al., 2014; Edwards et al., 2016; Xue et al., 2018), parallel investigator-sponsored Phase 2 studies (Dimopoulos et al; 2018; Fischer et al., 2019; Lam et al., 2019), and Phase 2 and Phase 3 studies sponsored by Nightstar Therapeutics, now part of Biogen Inc.

10.4 Procedures for Recording Adverse Events

All AEs occurring up to 24 months of follow-up post intervention that are observed by the investigator or reported by the participant will be recorded following the procedure outlined in Section 7.3, whether or not attributed to study medication.

The following information will be recorded:

- Description
- Date of onset and end date
- Severity
- Assessment of relatedness to the IMP, surgical procedure, other suspect drug or device
- Action taken

Follow-up information should be provided as necessary. The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

AEs considered related to the study medication (as judged by a medically qualified investigator or the nominated clinician for safety review) will be followed either until resolution or until the event is considered stable.

It will be left to the investigator's clinical judgment to decide whether or not an AE is of sufficient severity to require the participant's removal from treatment. A participant may also voluntarily withdraw from treatment due to what the said participant perceives as an intolerable AE. In either case, the participant must undergo the assessments specified for the Early Termination Visit and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.

10.5 Categorisation of Decreases in Vision as Adverse Events

Logarithm of the minimum angle of resolution (logMAR) charts such, as the ETDRS chart, have been authorised by the U.S. Food and Drug Administration (FDA) for measurement of vision (as quantified by BCVA) in gene therapy trials for retinal disorders[3]. The ETDRS chart has been used in the REGENERATE study, as well as in the preceding Phase 1/2 study (Xue et al., 2018) and parallel Phase 2 studies (Dimopoulos et al; 2018; Fischer et al., 2019; Lam et al., 2019).

Each line of 5 letters on the ETDRS chart is equivalent to 0.1 logMAR. This the $\log_{10}$ value of the change in minutes of arc resolution. A drop in BCVA of 0.3 logMAR (3 lines/15 letters) represents a doubling of the visual angle, as $\log_{10}2$ equals 0.30 (rounded to 2 significant figures).

A number of studies evaluating the reliability of ETDRS charts for BCVA measurement, using mean 95% coefficient of repeatability ($CR_{.95}$) as a measure of test–retest reliability (Bland and Altman, 1986), have been undertaken in order to determine whether changes in BCVA reflect true clinical change or are attributable to measurement error alone. In general, a change in BCVA of 0.2 logMAR (2 lines/10 letters) or greater can be reliably distinguished from no change with the ETDRS chart, but a change of 0.1 logMAR or less cannot (Rosser et al., 2003). As a general rule of thumb, $CR_{.95}$ is 11 letters of BCVA for subjects with severe visual impairments (Kiser et al., 2005).

Although changes in BCVA greater than 2 lines/10 letters on the ETDRS chart are considered to be outside test-retest variability, the FDA stipulates that a gain (or loss) of at least 3 lines/15 letters, corresponding to a halving (or doubling) of the visual angle, is required in order to be considered clinically meaningful[4].

Decreases in vision meeting the criteria for seriousness, as set out in Table 3, should be categorised accordingly and reported as SAEs. An exception is made for decreases in BCVA caused by cataract[5], which should be reported as AEs.

[3] U.S. Food and Drug Administration. Human Gene Therapy for Retinal Disorders [Internet]. 2020 January 30 [cited 2020 July 02]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-retinal-disorders.

[4] *Ibid*., p.9.

[5] Cataract is a clouding of the lens in the eye that leads to a decrease in vision. The occurrence of cataract is remedied by a straightforward outpatient procedure involving removal of the cloudy lens and its replacement with an artificial lens. A recent study has shown that cataract surgery is effective in choroideremia patients, and without any specific risks (Edwards et al., 2015). Hence, if clinically indicated, participants in the REGENERATE study who develop cataract may undergo corrective surgery. If cataract surgery is performed, it should be carried out at least 4 weeks before the next scheduled visit.

10.6 Reporting Procedure for Serious Adverse Events

All SAEs must be reported on the study-specific SAE Report Form to the Oxford Clinical Trials Research Unit (OCTRU[6]) **within 24 hours** of the site study team becoming aware of the event. OCTRU will date stamp the form to mark the date of awareness of the SAE and perform an initial check of the report, request any additional information, and will pass it on to the nominated clinician without delay. Additional and further requested information (follow-up or corrections to the original case) will be detailed on a new SAE Report Form.

A printed copy of the relevant SAE Report Form should be faxed to 01865 231534. Alternatively, a scanned copy may be emailed to regenerate@eye.ox.ac.uk.

All SAE Report Forms will be processed by OCTRU as per the detailed instructions in OCTRU's SOP governing safety reporting for a clinical trial of an IMP (CTIMP).

10.7 Reporting Procedure for SUSARs

All SUSARs will be reported by OCTRU to the Medicines and Healthcare Products Regulatory Agency (MHRA) and to the relevant Research Ethics Committee (REC) and other parties as applicable. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

The lead investigators will be informed of all SUSARs.

10.8 Development Safety Update Reports

OCTRU, on behalf of the CI, will submit (in addition to the expedited reporting as detailed above) a Development Safety Update Report (DSUR) once a year throughout the study, or on request, to the MHRA, relevant REC, host NHS Trusts and Sponsor.

[6] OCTRU is the Clinical Trials Unit (CTU) overseeing this Phase 2 clinical trial.

11 STUDY OVERSIGHT

11.1 Trial Management Group

The Trial Management Group (TMG) consists of those individuals responsible for the operational management of the trial such as the lead investigators (CI, PI and assistant investigators), key members of the scientific and clinical team (scientists, clinicians, optometrists and research nurses), the clinical trial coordinator, and representatives from OCTRU (clinical trial managers, statisticians, quality assurance managers and regulatory advisers). The TMG will meet every 1-2 months throughout the lifetime of the Phase 2 study and will:

- Supervise the conduct and progress of the study, and adherence to the study protocol.
- Assess the safety and efficacy of the interventions during the study.
- Monitor the safety of the participants, and review safety data to look for any emerging trends including increases in severity or frequency of SAEs or SARs (which may require expedited reporting to the MHRA and relevant REC).
- Evaluate the quality of the study data.
- Review relevant information from other sources (e.g. related studies).
- Escalate any issues for concern to the Sponsor, specifically where the issue could compromise patient safety or the integrity of the study or quality of the study data.

11.2 Trial Steering Committee

The chair and members of the Trial Steering Committee (TSC) will be formally appointed by the Director of the Efficacy and Mechanism Evaluation (EME) Programme which is the programme funding this Phase 2 study. The TSC is an independent body responsible for overall supervision of this study on behalf of the Sponsor (the University of Oxford) and the Funder (the EME Programme) in order to ensure that:

- Progress is satisfactory and the study is adhering to its overall objectives as set out in the protocol.
- Patient safety is not being compromised.
- The study is being conducted in accordance with Good Clinical Practice (GCP) and the UK Clinical Trial Regulations.

Decisions about continuation or termination of the study or substantial amendments to the protocol are usually the responsibility of the TSC, and the TSC will provide information and advice to the Sponsor, Funder and TMG in this regard.

Meetings of the TSC will take place annually, or at shorter intervals if required. Representatives of the Sponsor and the Funder will be invited to all TSC meetings.

11.3 Data Monitoring Committee

The Data Monitoring Committee (DMC) is an independent committee whose members consist of experts in fields such as ophthalmology, vitreoretinal surgery and gene therapy. The members of the DMC will be approved by the Funder (the EME Programme) prior to their appointment.

The DMC is responsible for review and analysis of the accruing study data (including trends in AEs), as well as data emerging from other related studies, in order to determine whether the safety of the study participants is at risk. If this is determined to be the case, the DMC will make recommendations to the TSC of the appropriate measures to be taken, e.g. a modification of the protocol or the termination of the study.

Meetings of the DMC will take place annually, or at shorter intervals if required, and subsequent reports will be forwarded to the TSC.

12 STATISTICS

12.1 Number of Participants

Thirty participants will be enrolled into this study. The participants will fall in one or both of the following cohorts:

- Cohort 1 will include all participants and will make comparisons of visual function in the treated eye before and after surgery in relation to vector dose per unit area of retina.
- Cohort 2 will be the subset of participants in whom treatment has been randomised and will make comparisons to the fellow untreated (control) eye with regard to the rate of anatomical degeneration.

No formal sample size calculations have been performed. The AAV2.REP1 vector is very expensive when manufactured to GMP standards and, in consequence, participant numbers have necessarily been dependent on the level of funding obtained. A sample size of 30 participants was deemed sufficiently statistically powered to show efficacy while still keeping funding requirements below £2 million.

12.2 Statistical Plan

12.2.1 Statistical Analysis of Data from Cohort 1

Summary statistics of each assessed variable will be presented for treated eyes versus control eyes (untreated fellow eyes). For these data, proportions per category, mean and standard deviation will be given – no formal statistical comparisons will be performed, i.e. no *p*-values and no confidence intervals will be computed.

12.2.2 Statistical Analysis of Data from Cohort 2

Data involving a comparison of an assessed variable between the treated and untreated eyes (of each participant) will be estimated as the difference between the eyes (with a 95% confidence interval), using analysis of covariance to compare assessed values adjusted for baseline values. Analysis of covariance has been recommended by Nash et al. (2014) as being much preferable to simple analysis of change from baseline; particularly for this study which will include some participants with asymmetric disease.

12.2.3 Meta-analysis of Data

Other studies are expected to be run with a similar protocol in other countries. These studies will be subject to their own regulatory and ethical approval. A meta-analysis of individual participant data from these studies is planned. A separate statistical analysis plan describing the details of the meta-analysis will be developed.

13 DATA MANAGEMENT

13.1 Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data).

13.2 Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host NHS Trusts and the MHRA to permit study-related monitoring, audits and inspections.

13.3 Data Recording and Record Keeping

Trial data (including data for AEs – see Section 7.3) will be recorded in paper CRFs which will be stored securely at each of the participating research sites. The data captured in the paper CRFs will be entered by site staff in a timely fashion into the trial database which is hosted securely off site.

A participant will be identified solely by a study ID in the CRF – the name and any other identifying details of a participant will not be included.

13.4 Ownership of Data

The electronic database used for the CRF is managed by Biogen Inc. Note that the data stored on the electronic database remains the property of the Sponsor (University of Oxford). The Sponsor has licenced the use of AAV2.REP1 to Biogen Inc. for its future development as a retinal gene therapy, as has likewise licenced to the use of data collected in this clinical trial to Biogen Inc.

13.5 Retention of Data

The data collected during this study will be used to support ongoing research and a future application for regulatory approval by Biogen Inc. Therefore the data collected during this study may be stored indefinitely. The data may also be shared anonymously with other researchers, including commercial organisations, in agreement with Biogen Inc.

14 QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with relevant regulations, GCP, the current approved protocol and study-specific SOPs, and local hospital site policies and guidelines.

A risk assessment will be performed and documented before the study starts. Based on the risk assessment, a monitoring plan will be drafted to ensure a proportionate approach to study management and monitoring. The monitoring plan will include central monitoring activities.

15 SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree –

a. "the safety or physical or mental integrity of the subjects of the trial; or
b. "the scientific value of the trial".

In the event that a serious breach is suspected, OCTRU's SOP governing serious breaches will be followed and the Sponsor will be contacted within 1 working day from awareness of the breach. In collaboration with the CI, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the relevant REC committee, the MHRA and other appropriate parties within 7 days.

16 ETHICAL AND REGULATORY CONSIDERATIONS

16.1 Declaration of Helsinki

The CI will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki (Fortaleza, Brazil, October 2013).

16.2 Guidelines for GCP

The CI will ensure that this study is conducted in accordance with relevant regulations and in compliance with GCP.

16.3 Approvals

The study protocol, ICF, PIS and GP covering letter will be submitted to the Sponsor, the relevant REC, and the MHRA for written approval.

The CI will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.4 Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress Report to the relevant REC and the Sponsor. In addition, an End of Study notification and final report will be submitted to the MHRA, the relevant REC and the Sponsor.

16.5 Participant Confidentiality

The study will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which requires data to be anonymised as soon as it is practical to do so, and for all documents (whether printed or electronic) to be stored securely and accessible only by authorised personnel.

In order to preserve the anonymity of participants, they will be referred to solely by a study ID (and not by their names or any other identifying details) in all study-related documents, with the exception of their signed ICFs.

16.6 Expenses and Benefits

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

17 FINANCE AND INSURANCE

17.1 Funding

This Phase 2 clinical trial of a gene therapy for choroideremia is supported by a £1.6 million grant from the EME Programme which is jointly funded by the Medical Research Council (MRC) and the National Institute for Health Research (NIHR). The EME Programme is managed by the NIHR Evaluation, Trials and Studies Coordinating Centre (NETSCC) based at the University of Southampton. The EME Programme funds clinical studies to test interventions where proof of concept has already been demonstrated, allowing their progress through early clinical trials and on to larger, later clinical trials.

17.2 Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). Product liability for the IMP will be provided by Biogen Inc. NHS indemnity operates in respect of the clinical treatment which is provided.

18 PUBLICATION POLICY

The CI will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study and will retain final editorial control. The authors shall acknowledge that the study was carried out with support from the EME Programme and all other funding bodies that have contributed to the AAV2.REP1 research programme.

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20 APPENDIX A: AMENDMENT HISTORY

<table>
<tr><th>Version Number</th><th>Issue Date</th><th>Author/ Reviewer</th><th>Significant Changes from Previous Version</th></tr>
<tr><td>1.0</td><td>22 July 2015</td><td>Dr Marco Bellini</td><td>Not applicable for the first issue</td></tr>
<tr><td>2.0</td><td>19 October 2015</td><td>Dr Marco Bellini</td><td>SYNOPSIS, Exclusion Criteria: the text ‘if relevant’ is deleted from the end of the fifth exclusion criterion ‘Unwillingness to use barrier contraception methods for a period of three months following gene therapy surgery, if relevant.’
SECTION 6.4, Exclusion Criteria: the text ‘if relevant’ is deleted from the end of the fifth exclusion criterion ‘Unwillingness to use barrier contraception methods for a period of three months following gene therapy surgery, if relevant.’</td></tr>
<tr><td>3.0</td><td>14 November 2016</td><td>Dr Marco Bellini</td><td>KEY TRIAL CONTACTS: Professor Lyndon da Cruz is inserted as Principal Investigator; Professor Andrew Webster is re-designated as Investigator and Co-applicant.
SECTION 4.6, Clinical Trial of a Retinal Gene Therapy for Choroideremia: ‘Edwards et al., 2016’ is inserted as a new reference after ‘MacLaren et al., 2014’ in the first paragraph.
SECTION 6.6, Informed Consent: the second and third paragraphs are deleted and replaced with the text ‘The decision about which eye to treat will be made on clinical grounds and will generally be the worse eye affected in cases where BCVA differs between the two eyes by 2 lines or more of ETDRS letters. The eye to be treated will be randomised in cases where the degeneration is relatively symmetrical between the two eyes, defined as:
a difference in BCVA of no more than 1 line of ETDRS letters, and
no more than 25% difference in the area of surviving RPE as measured by fundus autofluorescence.</td></tr>
</table>

			The following treatment options will be discussed in detail and agreed with each prospective participant as part of the informed consent process: • Prospective participants having non-symmetrical retinal degeneration will be allocated to the non-randomised arm. The treated eye will generally be the worse eye. • Prospective participants having relatively symmetrical retinal degeneration will be allocated to the randomised arm. It will be made clear in discussion with the prospective participants that they will not know which eye will be selected for treatment ahead of surgery. This requirement is necessary in order to avoid the possibility of selection bias. Prospective participants will be allowed as much time as wished to consider the information provided and, if necessary, to consult with their general practitioner (GP) or other independent parties so as to make an informed decision about their participation in the study. Another meeting with the investigator will be scheduled if more time for deliberation is required by the prospective participant.' • SECTION 7.3, Occurrence of Adverse Events during the Study: the paragraph is deleted and replaced with the text 'Any Adverse Event, irrespective of its perceived relationship to the AAV2.REP1 vector and/or the surgical procedure, will be captured in the participant's medical records. The relevant data will be recorded in the appropriate paper Case Report Form (CRF) and entered into the trial database following the general procedure for recording of trial data described in Section 13.3. The relevant safety procedures will be followed as detailed in Section 10'. • SECTION 7.4.1, Schedule of Study Visits: the text 'in Section 8.6' is deleted. • SECTION 7.4.2, Visit 1 (Screening): the text 'in Section 8.6' in the first paragraph is deleted; the text '2 weeks' in the third paragraph is deleted and replaced with the text '8 weeks'.

			• SECTION 7.4.3, Visit 2 (Surgery, Day 0): the text '2 weeks' in the first paragraph is deleted and replaced with the text '8 weeks'; the text ', and a full ophthalmic examination will occur prior to surgery to exclude any changes that might have occurred since the screening visit (see Table 2)' is deleted from the end of the second paragraph.
			• SECTION 7.4.4, Visits 3-11 (Follow-Up, Day 1 to Month 24): the text 'in Section 8.6' in the last paragraph is deleted.
			• SECTION 7.4.5, Unscheduled Visit: the text 'in Section 8.6' is deleted.
			• SECTION 8.1, Study Assessments: the text 'held in the Trial Master File (TMF), with the exception of Pattern ERG (PERG) for which the published standard protocol (2012 update) of the International Society for Clinical Electrophysiology of Vision (ISCEV) will be followed (Bach et al., 2013)' is deleted from the end of the first paragraph and replaced with the text 'Copies of the relevant SOPs will be retained in the Trial Master File (TMF) and ISFs.'; the text 'PERG' in Table 1 is deleted and replaced with the text 'Pattern ERG (PERG)'.
			• SECTION 8.4.1, Collection and Processing of Biological Samples: the text 'in Section 8.6' in the first paragraph is deleted.
			• SECTION 8.5, Location of Study Assessments: the text 'Section 15.6' is deleted and replaced with the text 'Section 16.6'.
			• TABLE 2, Schedule of visits and visit-specific assessments: existing table is deleted and replaced with a new table having the following changes to the schedule of assessments –
			  ○ Permitted time frame between Visit 1 (Screening) and Visit 2 (Day 0, Surgery) extended from 2 weeks to 8 weeks.
			  ○ Full ophthalmic examination dropped at Visit 2 (Day 0, Surgery).
			  ○ Randomisation of the treated eye (if relevant) specified as occurring at Visit 2 (Day 0, Surgery).

			○ Viral shedding assessments at Visit 6 (Month 3), Visit 7 (Month 6), Visit 9 (Month 12) and Visit 11 (Month 24) are dropped. ○ Immunology assessments (com-prising immunochemistry and immunoassay assessments) at Visit 9 (Month 12) and Visit 11 (Month 24) are dropped. ○ Colour vision assessment introduced at the Early Termination Visit. • SECTION 9.2, IMP Dose: 'Edwards et al., 2016' is inserted as a new reference after 'MacLaren et al., 2014' in the last paragraph. • SECTION 10.4, Procedures for Recording Adverse Events: the text 'on the CRF' in the first paragraph is deleted and replaced with the text 'following the procedure outlined in Section 7.3'. • SECTION 10.5, Reporting Procedure for Serious Adverse Events: the text 'An electronic SAE Report Form tool will be incorporated into the electronic CRF to be used in this study. The sites will enter data directly into the electronic SAE Report Form tool, i.e. by remote data entry.' is deleted as these instructions for recording AEs are already outlined in Section 7.3 and Section 10.4. • SECTION 13.3, Data Recording and Record Keeping: the first and second paragraphs are deleted and replaced with the text 'Trial data (including data for AEs – see Section 7.3) will be recorded in paper CRFs which will be stored securely at each of the participating research sites. The data captured in the paper CRFs will be entered by site staff in a timely fashion into the trial database which is hosted securely off site. A participant will be identified solely by a study ID in the CRF – the name and any other identifying details of a participant will not be included.' • SECTION 13.4, Ownership of Data: the first sentence 'The electronic CRF for this study is being developed and managed by NightstaRx Limited.' is deleted and replaced with the text 'The electronic database used for the CRF is managed by NightstaRx Limited.'; the text 'CRF' in the second sentence is deleted and replaced with the text 'database'.

			• REFERENCES: the reference 'Bach M, Brigell MG, Hawlina M, Holder GE, Johnson MA, McCulloch DL, Meigen T, Viswanathan S. ISCEV standard for clinical pattern electroretinography (PERG): 2012 update. Doc Ophthalmol. 2013;126(1):1-7.' is deleted; the reference 'Edwards TL, Jolly JK, Groppe M, Barnard AR, Cottriall CL, Tolmachova T, Black GC, Webster AR, Lotery AJ, Holder GE, Xue K, Downes SM, Simunovic MP, Seabra MC, MacLaren RE. Visual Acuity after Retinal Gene Therapy for Choroideremia. N Engl J Med. 2016;374(20):1996-8.' is inserted.
4.0	04 January 2017	Dr Marco Bellini	• TABLE 2, Schedule of visits and visit-specific assessments: existing table is deleted and replaced with a new table having the following changes to the schedule of assessments – ○ Vital signs: new assessment added at Visit 5 (Month 1) while Visits 6-11 (Month 3, Month 6, Month 9, Month 12, Month 18 and Month 24) are dropped. ○ Blood chemistry: new assessment added at Visit 5 (Month 1) while Visit 6 (Month 3), Visit 7 (Month 6), Visit 9 (Month 12) and Visit 11 (Month 24) are dropped. ○ Immunology assessments (com-prising immunochemistry and immunoassay assessments) at Visit 4 (Day 7) and Visit 6 (Month 3) are dropped. ○ Fundus photography assessment at Visit 10 (Month 18) is dropped. ○ Refractive error and BCVA assessments at Visit 3 (Day 1) are dropped.
5.0	15 March 2017	Dr Marco Bellini	• SYNOPSIS, Inclusion Criteria: the text 'equal to or worse than 6/6 (20/20; Decimal 1.0; LogMAR 0) but' is deleted from the fifth exclusion criterion 'Best corrected visual acuity equal to or worse than 6/6 (20/20; Decimal 1.0; LogMAR 0) but better than or equal to 6/60 (20/200; Decimal 0.1; LogMAR 1.0) in the study eye.' • SECTION 6.3, Inclusion Criteria: the text 'equal to or worse than 6/6 (20/20; Decimal 1.0; LogMAR 0) but' is deleted from the fifth exclusion criterion 'Best

			corrected visual acuity equal to or worse than 6/6 (20/20; Decimal 1.0; LogMAR 0) but better than or equal to 6/60 (20/200; Decimal 0.1; LogMAR 1.0) in the study eye.'
6.0	04 October 2017	Dr Marco Bellini	• Conflict of Interest Statement: the paragraph is deleted and is replaced with the text 'The Chief Investigator of the study, Professor Robert MacLaren, is the academic founder of Nightstar Therapeutics, a gene therapy company established in 2014 by the University of Oxford with funding from Syncona, a subsidiary of the Wellcome Trust. Through Oxford University Innovation, the technology transfer arm of the University of Oxford, Professor MacLaren is a named inventor on patents licensed to Nightstar Therapeutics. Professor MacLaren is also a non-executive director of Nightstar Therapeutics and he owns shares in the company. Although the REGENERATE trial is sponsored by the University of Oxford, the Department of Health which funds the trial has entered into an agreement with Nightstar Therapeutics in regard to the sharing of data from the REGENERATE trial for potential future regulatory approvals. Professor MacLaren therefore needs to declare this potential conflict of interest to all participants in the study. The REGENERATE trial is, however, overseen by an independent data and safety monitoring committee, an independent trial steering committee and the National Institute for Health Research's Efficacy and Mechanism Evaluation Programme.' • SYNOPSIS, Exclusion Criteria: the text 'minimum of 3 weeks' is deleted from the end of the fourth exclusion criterion 'Inability to take systemic prednisolone for a minimum of 3 weeks' and is replaced with the text 'period of 45 days.' • SECTION 6.4, Exclusion Criteria: the text 'minimum of 3 weeks' is deleted from the end of the fourth exclusion criterion 'Inability to take systemic prednisolone for a minimum of 3 weeks' and is replaced with the text 'period of 45 days.'

- SECTION 7.4.2, Visit 1 (Screening): the text in the section entitled 'Prednisolone' is deleted and replaced with the text 'In order to minimise postoperative inflammation, participants will be given a 45 day course of oral prednisolone, starting at a daily dose of 1 mg per kg of body weight (rounded to the nearest multiple of 5 mg) and gradually tapering off to 10 mg and 5 mg in the second last and last weeks:

Days	**Daily dose/ kg**	**Daily dose (rounded to nearest multiple of 5 mg) for adult weighing:**				
		60 kg	**70 kg**	**80 kg**	**90 kg**	**100 kg**
3 days before surgery	1 mg/kg	60 mg	70 mg	80 mg	90 mg	100 mg
7 days after surgery	1 mg/kg	60 mg	70 mg	80 mg	90 mg	100 mg
7 days	0.75 mg/kg	45 mg	55 mg	60 mg	70 mg	75 mg
7 days	0.5 mg/kg	30 mg	35 mg	40 mg	45 mg	50 mg
7 days	0.25 mg/kg	15 mg	20 mg	20 mg	25 mg	25 mg
7 days	0.1 mg/kg	10 mg	10 mg	10 mg	10 mg	10 mg
7 days	0.05 mg/kg	5 mg	5 mg	5 mg	5 mg	5 mg

  Prednisolone tablets will be taken once a day, preferably in the morning with food. If the day of surgery is regarded as Day 0, then the 45 day course starts on Day −2 and ends on Day 42.'; the text '21 days' in the section entitled 'Omeprazole' is deleted and replaced with the text '45 days'.
- TABLE 2, Schedule of visits and visit-specific assessments: the following changes are made to the schedule of assessments –
  - Row entitled 'Prednisolone/ omeprazole administration': the text 'Day 19' is deleted and replaced with the text 'Day 42'.
  - Footnote 'e': the text '21 days' is deleted and replaced with the text '45 days'; the text 'Day 19' is deleted and replaced with the text 'Day 42'.

			• SECTION 9.3, Storage of IMP: the paragraph is deleted and replaced with the text 'The IMP (AAV2.REP1 vector suspension) is stored at −60°C or lower prior to use. Shortly before surgery, a sufficient number of vials of the AAV2.REP1 vector suspension (corresponding to a total volume of no less than 0.2 ml) are withdrawn from storage and transported to the operating theatre, in accordance with local hospital policies and procedures in force at each site. The AAV2.REP1 vector suspension is permitted to thaw en route to the operating theatre. Should surgery be delayed for whatever reason, the AAV2.REP1 vector suspension may be stored temporarily at 4°C in a refrigerator in the vicinity of the operating theatre until required. Stability tests have shown that the vector can be kept at 4°C for at least one week.' • SECTION 13.4, Ownership of Data: all occurrences of the text 'NightstaRx Limited' are deleted and replaced with the text 'Nightstar Therapeutics'. • SECTION 17.2, Insurance: the text 'NightstaRx Limited' is deleted and replaced with the text 'Nightstar Therapeutics'.

7.0	20 November 2018	Dr Marco Bellini	• Conflict of Interest Statement: the paragraph is deleted and is replaced with the text 'The Chief Investigator of the study, Professor Robert MacLaren, is a consultant to several gene therapy companies, including Biogen Inc., which is developing the choroideremia gene therapy programme internationally. He is also employed by the University of Oxford which owns two patents relevant to choroideremia gene therapy on which he is a named inventor. He does not however receive any payments for his role in this study and does not hold shares, incentives or options in any of the companies involved.'
			• KEY TRIAL CONTACTS, Chief Investigator, Professor Robert MacLaren: the following changes are made –
			  ○ The existing telephone number '223380' is deleted and is replaced with the new telephone number '228974'.
			  ○ The text 'Sue Ball, ' is deleted.

			○ The existing text 'enquiries@eye.ox.ac.uk' is deleted and is replaced with the new text 'maclaren@eye.ox.ac.uk (Personal Assistant)'. • KEY TRIAL CONTACTS, Principal Investigator, Professor Lyndon da Cruz: the following changes are made – ○ The text 'Hilary Newman, ' is deleted. ○ The text 'lyndon.dacruz@moorfields.nhs.uk' is deleted and is replaced with the new text 'lyndon.dacruz1@nhs.net'. • KEY TRIAL CONTACTS, Investigator and Co-applicant, Professor Andrew Webster: this co-applicant is no longer involved with this study due to work commitments, and has accordingly been removed from this list. • KEY TRIAL CONTACTS, Co-applicant, Professor Doug Altman: this co-applicant is unfortunately now deceased, and has accordingly been removed from this list. • KEY TRIAL CONTACTS, Representative of Sponsor: the text ', Block 60 Churchill Hospital Old Road Oxford OX3 7LE' is deleted and is replaced with the text 'Boundary Brook House Churchill Drive Oxford OX3 7LQ'. • KEY TRIAL CONTACTS, Representative of Clinical Trials Unit: the following changes are made – ○ The text 'Dr Surjeet Singh' is deleted and is replaced with the text 'Mr Damian Haywood'. ○ The text 'surjeet.singh@nds.ox.ac.uk' is deleted and is replaced with the text 'damian.haywood@nds.ox.ac.uk'. • KEY TRIAL CONTACTS, Chief Scientist, Dr Alun Barnard: this member of the trial management team has left the employment of the University of Oxford, and has accordingly been removed from this list. • SECTION 3, ABBREVIATIONS: the following changes are made – ○ A new row entitled 'CR_{95}' is created, and the text '95% coefficient of repeatability' is inserted. ○ A new row entitled 'FDA' is created, and the text 'U.S. Food and Drug Administration' is inserted.

			○ The text 'Chart measuring visual acuity as the ' is deleted in the row entitled 'logMAR'. • SECTION 4.6, Clinical Trial of a Retinal Gene Therapy for Choroideremia: the following changes are made – ○ The text 'Trial' is deleted in the section title and is replaced with the text 'Trials'. ○ The first and second paragraphs are deleted and replaced with the following text: 'In 2011, a Phase 1/2 clinical trial (ClinicalTrials.gov: NCT01461213; EudraCT: 2009-014617-27) of a gene therapy for choroideremia was commenced, using a modified AAV2 vector having a CAG promoter and the REP1 cDNA sequence (i.e. the exonic regions of the *CHM* gene encoding REP1), designated as AAV2.REP1. Further to the positive initial results from the first 6 choroideremia patients treated with the AAV2.REP1 vector (MacLaren et al., 2014), followed by publication of data showing that the effect of gene therapy was sustained indefinitely (Edwards et al., 2016), regulatory approval of this Phase 2 clinical trial (ClinicalTrials.gov: NCT02407678; EudraCT: 2015-001383-18) was obtained to test the AAV2.REP1 vector in an additional 30 choroideremia patients. Subsequently, the 24 month results of all 14 choroideremia patients treated the original Phase 1/2 clinical trial have been published (Xue et al, 2018). The median best corrected visual acuity [Footnote: Best corrected visual acuity = visual acuity measured after refractive errors in the eye, such as myopia, hypertropia, astigmatism and presbyopia, have been corrected with eyeglasses or contact lenses where necessary.] (BCVA), measured using Early Treatment Diabetic Retinopathy Study (ETDRS) charts, improved by 4.5 letters across all 14 treated eyes, while declining by −1.5 letters across all 14 untreated eyes. The trial

			thus met its primary endpoint of improving vision following gene therapy compared to untreated fellow eyes, despite any potential adverse effects of retinal detachment. In 12 out of 14 patients, the retinal gene therapy led to recovery of visual acuity in all eyes and variable degrees of acuity gains that generally occurred within 6 months of treatment and were sustained up to 5 years. Results published from three parallel Phase 2 clinical trials testing the same batch of AAV2.REP1 vector have all shown good safety results from subretinal administration, and gains of more than 3 lines of vision (measured with ETDRS charts) in 2 of the 18 patients enrolled (Dimopoulos et al; 2018; Fischer et al., 2019; Lam et al., 2019).'. • TABLE 2, Schedule of visits and visit-specific assessments: the existing text in footnote 'c', pertaining to assessments to be conducted at an unscheduled visit, is replaced with the new text 'Full ophthalmic examination and BCVA assessment should be conducted as a minimum. Microperimetry, fundus autofluorescence and OCT assessments, as well as monitoring of concomitant medication and adverse events, may also be performed if clinically required.'. • SECTION 9.1, Description of the Investigational Medicinal Product: the existing text 'University of Oxford' in the first paragraph is replaced with the new text 'Biogen Inc.', and the existing text 'accompanying Investigator's Brochure (IB)' in the last paragraph is replaced with the new text 'Investigator's Brochure (IB) prepared by Nightstar Therapeutics, now part of Biogen Inc.'. • SECTION 9.2, IMP Dose: the text '; Xue et al., 2018' is inserted as an additional reference in the last paragraph. • SECTION 10.3, Expectedness: the existing text 'Phase 1 study.' at the end of this section is replaced with the new text 'Phase 1/2 study (MacLaren et al., 2014;

			Edwards et al., 2016; Xue et al., 2018), parallel investigator-sponsored Phase 2 studies (Dimopoulos et al; 2018; Fischer et al., 2019; Lam et al., 2019), and Phase 2 and Phase 3 studies sponsored by Nightstar Therapeutics, now part of Biogen Inc.'. • A new SECTION 10.5, entitled 'Categorisation of Decreases in Vision as Adverse Events' is created, and the following text is inserted: 'Logarithm of the minimum angle of resolution (logMAR) charts such, as the ETDRS chart, have been authorised by the U.S. Food and Drug Administration (FDA) for measurement of vision (as quantified by BCVA) in gene therapy trials for retinal disorders [Footnote: U.S. Food and Drug Administration. Human Gene Therapy for Retinal Disorders [Internet]. 2020 January 30 [cited 2020 July 02]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-retinal-disorders.]. The ETDRS chart has been used in the REGENERATE study, as well as in the preceding Phase 1/2 study (Xue et al., 2018) and parallel Phase 2 studies (Dimopoulos et al; 2018; Fischer et al., 2019; Lam et al., 2019). Each line of 5 letters on the ETDRS chart is equivalent to 0.1 logMAR. This the $\log_{10}$ value of the change in minutes of arc resolution. A drop in BCVA of 0.3 logMAR (3 lines/15 letters) represents a doubling of the visual angle, as $\log_{10}2$ equals 0.30 (rounded to 2 significant figures). A number of studies evaluating the reliability of ETDRS charts for BCVA measurement, using mean 95% coefficient of repeatability ($CR_{.95}$) as a measure of test–retest reliability (Bland and Altman, 1986), have been undertaken in order to determine whether changes in BCVA reflect true clinical change or are attributable to measurement error alone. In general, a change in BCVA of 0.2 logMAR (2 lines/10 letters) or greater can be reliably distinguished from no change with the ETDRS chart, but a change of 0.1 logMAR or less cannot (Rosser et al., 2003). As a general rule of thumb, $CR_{.95}$ is

			11 letters of BCVA for subjects with severe visual impairments (Kiser et al., 2005). Although changes in BCVA greater than 2 lines/10 letters on the ETDRS chart are considered to be outside test-retest variability, the FDA stipulates that a gain (or loss) of at least 3 lines/15 letters, corresponding to a halving (or doubling) of the visual angle, is required in order to be considered clinically meaningful [Footnote: *Ibid*., p.9.]. Decreases in vision meeting the criteria for seriousness, as set out in Table 3, should be categorised accordingly and reported as SAEs. An exception is made for decreases in BCVA caused by cataract [Footnote: Cataract is a clouding of the lens in the eye that leads to a decrease in vision, and can develop as a result of the vitrectomy procedure. The occurrence of cataract is remedied by a straightforward surgical procedure involving removal of the cloudy lens and its replacement with an artificial lens. A recent study has shown that cataract surgery is effective in choroideremia patients, and without any specific risks (Edwards et al., 2015). Hence, if clinically indicated, participants in the REGENERATE study who develop cataract may undergo corrective surgery. If cataract surgery is performed, it should be carried out at least 4 weeks before the next scheduled visit.], which should be reported as AEs.'. • SECTION 13.4, Ownership of Data: all occurrences of the text 'Nightstar Therapeutics' are deleted and replaced with the text 'Biogen Inc.'. • SECTION 13.5, Retention of Data: the existing text 'potential Phase 3 clinical trial with the ultimate goal of developing a licensed medicinal product' is replaced with the new text 'future application for regulatory approval by Biogen Inc.', and new text ', in agreement with Biogen Inc.' is inserted at the end of this section. • SECTION 16.5 Participant Confidentiality: the existing text 'Data Protection Act' is replaced with the new text

			'General Data Protection Regulation (GDPR) and Data Protection Act 2018'. • SECTION 17.2 Insurance: the existing text 'Nightstar Therapeutics' is replaced with the new text 'Biogen Inc.'. • REFERENCES: the following references are inserted – ○ 'Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476): 307-10.' ○ 'Dimopoulos IS, Hoang SC, Radziwon A, Binczyk NM, Seabra MC, MacLaren RE, Somani R, Tennant MTS, MacDonald IM. Two-Year Results After AAV2-Mediated Gene Therapy for Choroideremia: The Alberta Experience. Am J Ophthalmol. 2018;193:130-142.' ○ 'Edwards TL, Groppe M, MacLaren RE. Outcomes following cataract surgery in choroideremia. Eye. 2015;29(4):460-4.' ○ 'Fischer MD, Ochakovski GA, Beier B, Seitz IP, Vaheb Y, Kortuem C, Reichel FFL, Kuehlewein L, Kahle NA, Peters T, Girach A, Zrenner E, Ueffing M, MacLaren RE, Bartz-Schmidt KU, Wilhelm B. Efficacy and Safety of Retinal Gene Therapy Using Adeno-Associated Virus Vector for Patients With Choroideremia: A Randomized Clinical Trial. JAMA Ophthalmol. 2019;137(11): 1247-1254.' ○ 'Kiser AK, Mladenovich D, Eshraghi F, Bourdeau D, Dagnelie G. Reliability and consistency of visual acuity and contrast sensitivity measures in advanced eye disease. Optom Vis Sci. 2005;82(11):946-54.' ○ 'Lam BL, Davis JL, Gregori NZ, MacLaren RE, Girach A, Verriotto JD, Rodriguez B, Rosa PR, Zhang X, Feuer WJ.Choroideremia Gene Therapy Phase 2 Clinical Trial: 24-Month Results. Am J Ophthalmol. 2019;197:65-73.' ○ 'Rosser DA, Cousens SN, Murdoch IE, Fitzke FW, Laidlaw DA. How sensitive to clinical change are

			ETDRS logMAR visual acuity measurements? Invest Ophthalmol Vis Sci. 2003;44(8):3278-81.’ ○ ‘Xue K, Jolly JK, Barnard AR, Rudenko A, Salvetti AP, Patrício MI, Edwards TL, Groppe M, Orlans HO, Tolmachova T, Black GC, Webster AR, Lotery AJ, Holder GE, Downes SM, Seabra MC, MacLaren RE. Beneficial effects on vision in patients undergoing retinal gene therapy for choroideremia. Nat Med. 2018;24(10):1507-1512.’

All trial protocol amendments will be submitted to the Sponsor for approval prior to submission to the relevant REC or the MHRA.