CLINICAL STUDY PROTOCOL
NSR-REP-02 (273CH203)

AAV2-REP1

An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)

GEMINI Study

INDICATION:	Choroideremia (CHM)
STUDY PHASE:	2
EUDRACT NUMBER:	2017-002395-75
CLINICAL TRIAL NUMBER:	NCT03507686
INN:	Timrepigene emparvovec
SPONSOR:	NightstaRx Ltd. (A Biogen Company) Biogen Idec Research Limited Innovation House 70 Norden Road Maidenhead, Berkshire SL6 4AY United Kingdom
Final Protocol Date:	4 Jul 2017
Amendment 1, Version 2	17 May 2018
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Amendment 4.0, Version 5.0	30 Apr 2020
Amendment 5.0, Version 6.0	12 Nov 2020

Version 6 of protocol NSR-REP-02 supersedes Version 5, dated 30 Apr 2020.

SPONSOR APPROVAL PAGE

Clinical Study Protocol Number: NSR-REP-02 (273CH203)

Protocol Title: An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)

Protocol Date: 12 Nov 2020

Approved By: [redacted], MD
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Protocol No. NSR-REP-02 was approved by:

[redacted]

[redacted], MD

[redacted]

Biogen

INVESTIGATOR'S SIGNATURE PAGE

Clinical Study Protocol Number: NSR-REP-02 (273CH203)

Protocol Title: An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)

Protocol Date: 12 Nov 2020

I have read the Investigator's Brochure for AAV2-REP1 and I have read Protocol NSR-REP-02 and agree to conduct the study as outlined and in compliance with the Declaration of Helsinki (where required), the International Council on Harmonisation (ICH) guideline for Good Clinical Practice (GCP), and all applicable local and federal regulatory requirements and state/local laws. I agree to maintain confidentiality of my subjects and all information received or developed in relation to this protocol.

Signed: Date:

Name
Title
Institution
City, State (as applicable), Country

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Name	Address	Telephone Number
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Additional contact information is available in the Site Operations Manual.

2 PROTOCOL SYNOPSIS

Name of Sponsor/Company: NightstaRx, Ltd	
Name of Test Product: AAV2-REP1	
Protocol Title: An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)	
Protocol Number: NSR-REP-02	
Study centres: The study will be conducted at approximately 5 study centres in Europe and United States.	
Study period (years): Estimated overall study duration: 12 months of follow-up for each eye	**Phase of development:** Phase 2
Study Objective: The objective of the study is to evaluate the safety of bilateral, sequential sub-retinal administration of a single dose of AAV2-REP1 in adult male subjects with choroideremia (CHM).	
Primary Endpoint: The safety of bilateral administration of AAV2-REP1 will be evaluated with the following safety measures: • Best corrected visual acuity (BCVA) as measured by the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart • Ophthalmic examination assessments (including intraocular pressure [IOP], slit lamp examination, lens opacity grading and dilated ophthalmoscopy) • Spectral domain optical coherence tomography (SD-OCT) • Fundus autofluorescence (AF) • Fundus photography • Microperimetry • Adverse event (AE) reporting • Vector shedding post-treatment • Immunogenicity sampling post-treatment • Vital signs **Secondary Efficacy Endpoints will include:** • Change from Baseline in BCVA as measured by the ETDRS chart • Change from Baseline in AF • Change from Baseline in SD-OCT • Change from Baseline in microperimetry	
Study Design: This is a multi-centre, open-label, prospective, 2-period, bilateral interventional safety study of AAV2-REP1 in adult male subjects with genetically confirmed CHM. The study will consist of a Screening Period followed by 2 treatment periods (Period 1 and Period 2) with up to 9 visits per period. During the Screening Period, subjects will be assessed for eligibility of both eyes. The Investigator will assign the order in which the eyes are treated (i.e., study eye 1 [SE1]- Period 1 and study eye 2 [SE2]- Period 2, respectively) with a number assigned to each eye. This will be done in collaboration with the subject; however, the worse eye will generally be selected for first treatment. Study eye selection and eligibility for surgery will then be confirmed collectively by the Patient Eligibility Review Committee, which includes the Investigator, a consulting surgeon, and the Sponsor (see the Patient Eligibility Review Plan). The estimated target interval between the surgical procedures of SE1 and SE2 will be determined at the Screening Visit. Each study eye will be followed for at least 12 months post-treatment, for up to 9 visits per treatment period: Visit 1 (Day 0, Injection Day Visit); Visit 2 (Day 1, post-operative follow-up visit); Visit 3 (Day 3 + 2 days, post-operative follow-up visit); Visit 4 (Day 7 -1/+2 days); Visit 5	

(Day 14 ± 3 days); Visit 6 (Month 1 ± 7 days); Visit 7 (Month 3 ± 14 days); Visit 8 (Month 6 ± 14 days) and Visit 9 (Month 12 ± 14 days; end of study visit).

At Period 1, Visit 1 (Day 0, Injection Day Visit for SE1), subjects will undergo a vitrectomy with retinal detachment and receive a sub-retinal injection of AAV2-REP1 in SE1. Visits 2-9 will then be conducted according to the Schedule of Study Procedures, unless Period 2 commences during this time, in which case, the study visits will follow the schedule for SE2. Ophthalmic assessments during Period 1, Visits 2-9 will be performed on both eyes.

The interval between SE1 and SE2 treatment is expected to vary among subjects from weeks to many months. While the interval will be decided on a case-by-case basis, an effort will be made to schedule varying treatment intervals in order to better characterise the immunological and safety profile of sequential treatment administration. Thus, approximately 20 subjects each will be treated with a short (<6 month), medium (6-12 months), or long (>12 month) surgery window between treatment of the first and second eye. Because the timing of the second surgery visit (Period 2, Visit 1) will vary among subjects, the duration of study Period 1 will also be variable.

The post-operative outcome and visual function of SE1 will inform the scheduling of Period 2. Signs of post-operative inflammation, or other post-operative sequelae, as judged by the Investigator, should be resolved in SE1 for the subject to continue to Period 2. After comprehensive evaluation of all safety assessments and visual functional tests of SE1 and SE2, the Investigator will determine the eligibility of the subject and decide the appropriate time to schedule SE2, Visit 1.

All baseline ophthalmic assessments must be conducted within 10 weeks prior to SE2 surgery (Period 2, Visit 1). Eligibility for SE2 surgery will then be confirmed collectively by the Patient Eligibility Review Committee, which includes the Investigator, a consulting surgeon, and the Sponsor (see the Patient Eligibility Review Plan). If, at that time, the BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the Patient Eligibility Review Committee: 1) the subject is found eligible by all other inclusion and exclusion criteria, and 2) the subject is considered an appropriate candidate who has potentially modifiable disease.

At Period 2, Visit 1 (Day 0, Injection Day Visit for SE2), subjects will undergo a vitrectomy with retinal detachment and sub-retinal injection of AAV2-REP1 in the contralateral, untreated eye (SE2). Post-treatment, subjects will no longer follow the Period 1 visit schedule, but will instead attend Period 2, Visits 2-9 according to the Schedule of Study Procedures. Ophthalmic assessments scheduled for Period 2, Visits 2-9 will be performed on both eyes.

Subjects will be assessed for safety and efficacy throughout the study; assessments are outlined in the Schedule of Study Procedures. Subjects who develop cataracts may undergo cataract surgery if deemed clinically necessary. If cataract surgery is performed, it should be carried out at least 4 weeks before Month 12 (Visit 9) for the respective eye. The cataract surgery timing must be discussed with the medical monitor prior to the procedure.

Number of Subjects (Planned): Approximately 60 subjects are planned for the study.

Inclusion Criteria: During the Screening Period, subjects will be found eligible for study participation if they meet all of the following inclusion criteria.

1. Are willing and able to give informed consent for participation in the study to have both eyes treated
2. Are male and ≥18 years of age
3. Have documentation of a genetically-confirmed diagnosis of CHM
4. Have active disease clinically visible within the macular region of both eyes
5. Have a BCVA of ≥34 ETDRS letters (20/200 or better Snellen acuity) in both eyes, or in the untreated eye, if the other eye was previously treated with AAV2-REP1*
   *If previously treated with AAV2-REP1 in an antecedent study, subjects may be eligible for participation following Sponsor approval.
6. For subjects who received treatment with AAV2-REP1 in an antecedent study, have biological samples available to complete an adequate immunology profile

Exclusion Criteria: At the Screening Visit, subjects will be found not eligible for study participation if they meet any of the following exclusion criteria.

1. Have a history of amblyopia or inflammatory disorder in either eye 2. Are unwilling to use barrier contraception methods, or abstain from sexual intercourse for a period of 3 months following treatment with AAV2-REP1 in either eye 3. Have had previous intraocular surgery performed within 3 months of the Screening Visit in either eye 4. Have any other significant ocular or non-ocular disease/disorder which, in the opinion of the Patient Eligibility Review Committee or Investigator, may either put the subjects at risk because of participation in the study, or may influence the results of the study, or the subject's ability to participate in the study. This includes but is not limited to a potential subject: • with a contraindication to oral corticosteroid (e.g. prednisolone/prednisone) • with clinically significant cataract in either eye • who, in the clinical opinion of the Patient Eligibility Review Committee or Investigator, is not an appropriate candidate for sub-retinal surgery (see the Patient Eligibility Review Plan) 5. Have participated in another research study involving an investigational product in the past 12 weeks or received a gene/cell-based therapy at any time previously, except if treated within in an antecedent study with AAV2-REP1.
Test Product, Dosage, and Mode of Administration: For each eye, subjects will undergo vitrectomy and retinal detachment and receive a sub-retinal injection of up to 0.1 mL of study drug containing 1×10^11 AAV2-REP1 genome particles (gp).
Reference Therapy (Comparator), Dosage, and Mode of Administration: Not Applicable.
Criteria for Evaluation: **Safety:** The safety evaluation will be based on BCVA (as measured by the ETDRS chart); full ophthalmic examination (including IOP, slit lamp examination, lens opacity grading and dilated ophthalmoscopy); SD-OCT; fundus AF; fundus photography; microperimetry; AE reporting; vector shedding and immunogenicity; and vital signs. **Efficacy:** The efficacy evaluation will be based on BCVA (as measured by the ETDRS chart); fundus AF; SD-OCT and microperimetry.
Statistical Methodology: No formal sample size calculation will be performed. Continuous variables will be summarised over time using descriptive statistics (i.e., mean, standard deviation, 95% confidence intervals [CI], median, first and third quartiles, fifth and ninety-fifth percentiles, minimum, and maximum). Categorical variables will be described over time using counts, percentages, and 95% CIs. Summaries will be tabulated by visit and eye. No formal statistical testing will be performed. AEs will be summarised by system organ class, preferred term, and eye. The number of eyes with an AE, as well as the number of events, will be summarised, by period and eye. Similar summaries will be produced for study drug/procedure-related AEs, AEs leading to discontinuation, and serious AEs. AEs will also be summarised by maximum severity, relationship to study drug/procedure, and time to onset and resolution. Vector shedding and immune response profiles will be described. The remaining safety evaluations will be analysed using descriptive statistics.

3 TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

LIST OF TABLES

LIST OF FIGURES

4 ABBREVIATIONS AND DEFINITIONS

Abbreviation or Term	Definition
AAV	adeno-associated virus
AAV2	AAV serotype 2
AAV2-REP1	adeno-associated viral vector (AAV2) encoding rab escort protein 1
AE	adverse event
AF	autofluorescence
BCVA	best corrected visual acuity
BGH-polyA	bovine growth hormone polyadenylation
BSS	balanced salt solution
CBA	chicken β actin
cDNA	complementary deoxyribonucleic acid
CHM	choroideremia
CI	confidence interval
CRC	central reading centre
CRO	contract research organisation
eCRF	electronic case report form
DMC	data monitoring committee
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
ET	early termination
ETDRS	Early Treatment of Diabetic Retinopathy Study
GCP	good clinical practice
gp	genome particle
GTMP	gene therapy medicinal product
ICH	International Council on Harmonisation
IEC	Independent Ethics Committee
IOP	intraocular pressure
IRB	Institutional Review Board
LOCS	lens opacities classification system
REP1	Rab escort protein 1
SAE	serious adverse event
SAP	statistical analysis plan
SD	study day
SD-OCT	spectral domain optical coherence tomography
SE1/SE2	study eye 1/study eye 2
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
UK	United Kingdom
VA	visual acuity
WPRE	woodchuck hepatitis post-transcriptional regulatory element

5 INTRODUCTION

5.1 Choroideremia

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

5.2 Gene Therapy with Adeno-Associated Viral (AAV) Vectors

Gene therapy is rapidly emerging as a promising approach for the treatment of incurable retinal diseases (Bryant, Duker et al. 2012, Boye, Boye et al. 2013). Gene therapy medicinal products (GTMPs) are a type of advanced therapy medicinal product defined as medicines derived from genes and cells (EMA 2015). GTMPs contain or consist of recombinant nucleic acid delivered via a viral vector to targeted cells. The central goal of gene therapy is to replace non-functional or defective genes with new genes that are fully functional so that the level of genetic expression can return to normal.

The eye is particularly well suited for gene therapy because of its accessibility, relative immune privilege status, small size, compartmentalisation and the presence of a fellow control eye (Boye, Boye et al. 2013). The eye is one of the few relatively immunologically-privileged sites in the body, so vectors used in gene therapy are unlikely to cause a significant systemic immune response. It has long been established that to maintain the transparent structures required for vision, the eye has conserved a number of adaptations that selectively diminish the immune response (Streilein 2003). For example, complement-fixing antibodies, neutrophils and macrophages are generally excluded from mitigation of an inflammatory response to limit the formation of opacities. Anatomically, the retina is protected by the blood-retinal barrier to minimise the circulation of immune cells (Streilein 2003). Studies have demonstrated that antigens introduced to the vitreous and sub-retinal space exhibit a reduced immune response due to the varied cytokine environment and the presence of molecules that suppress the immune system (Stein-Strelein and Watte 2007).

Given the defined volume of the eye, the amount of viral vector needed to achieve a therapeutic effect will likely be small, reducing the risk of toxicity and increasing the likelihood of being able to manufacture quantities of vector sufficient to treat the retina. The eye also allows for localised treatment without intravenous delivery, thus decreasing the chance of systemic absorption and toxicity. Finally, the effects of localised ocular treatments can be easily observed and monitored for efficacy and safety, something that cannot be readily done with systemic conditions (Bryant, Duker et al. 2012).

The most commonly used delivery system for retinal gene therapy is the adeno-associated virus (AAV), and numerous early phase clinical trials using AAV vectors are currently underway for potential treatment of various retinal diseases (Boye, Boye et al. 2013, MacLaren, Groppe et al. 2014, Edwards, Jolly et al. 2016). AAVs remain in the nucleus as episomes with no integration into the human genome, thereby decreasing the risk of insertional oncogenesis. AAVs allow for stable and long term transgene expression in different retinal cells, including photoreceptors, retinal pigment epithelium cells, ganglion and Müller cells (Vanderberghe and Auricchio 2012, Day, Byrne et al. 2014). Lastly, AAVs

elicit a minimal immune response as supported by emerging data from non-clinical and clinical studies.

One non-clinical study evaluated immunological and functional consequences of adeno-associated viral vector type 2 (AAV2) vector re-administration to the contralateral eye in dogs and non-human primates (Amado, Mingozzi et al. 2010). In this study, rAAV2-hRPE65v2 administered to the untreated, contralateral eye 15 days after the first injection was found to be both efficacious and safe. The study demonstrated that cell-mediated immune responses were benign, with only 1 of 10 animals in the study developing a persistent T cell immune response to AAV2, a response that was mediated by CD4+ T cells. Sequential bilateral injection caused minimal inflammation and improved visual function in affected animals. The authors concluded that sub-retinal re-administration of AAV2 is safe even in the setting of pre-existing immunity to the vector, a parameter that has been used to exclude patients from gene therapy trials. In another non-clinical study in rats, lack of apparent immunogenicity was observed following same-day bilateral administration of 6×10^9 genome particles (gp)/eye of adeno-associated viral vector (AAV2) encoding Rab escort protein 1 (AAV2-REP1) (CharlesRiverLabs 2015). This total antigen exposure following bilateral dosing is comparable to a unilateral dose of 1.2×10^10 gp, which equates to a 1.2×10^12 gp dose in humans. This provides a 12-fold immunogenic safety margin compared to the planned clinical dose of 1×10^11 gp.

In the clinical setting, repeat administration of AAV2-RPE65 to the contralateral eye of children and adults was also shown to be safe, irrespective of baseline immune status (Bennett, Wellman et al. 2016). In this study, 11 children and adults with Leber's congenital amaurosis received a single sub-retinal administration of AAV2-RPE65 at a dose of 1.5×10^11 gp (1.7-4.6 years after initial sub-retinal injection) to the contralateral, previously untreated eye. No adverse events (AEs) related to AAV2 vector were reported, and humoral and cell-mediated responses to AAV2 capsid and RPE65 transgene were benign in all patients. Two individuals who had high pre-existing neutralizing AAV2-antibodies did not experience an inflammatory response in either eye following contralateral eye administration. AAV2-RPE65, voretigene neparvovec-rzyl (LUXTURNA™, Spark Therapeutics, Inc), has been approved in the US and the EU as the first adeno-associated virus vector type 2 (AAV2)-based gene therapy indicated for the treatment of patients with confirmed bi-allelic RPE65 mutation-associated retinal dystrophy.

5.3 Study Rationale

CHM is incurable and treatment is supportive at best. NightstaRx is developing AAV2-REP1 as a potential GTMP for the treatment of CHM. AAV2-REP1 is a recombinant AAV2 particle encapsulating 1.962kB complementary deoxyribonucleic acid (cDNA) of the wild-type human REP1 gene. Non-clinical studies with AAV2-REP1 have been very promising (Tolmachova, Tolmachov et al. 2013). AAV2-REP1 expresses high levels of human REP1 protein, restores REP1 to human CHM fibroblasts, provides functional rescue of human CHM cells, expresses protein in the retina of CHM mice in vivo, and is non-toxic when over-expressed by one log unit. Further, over-expression of the human REP1 protein does not significantly affect retinal function (Tolmachova, Tolmachov et al. 2013).

In 2011, an investigator-sponsored, first-in-human study of AAV2-REP1 was initiated in adult male patients with CHM. The first 6 patients were treated with a single sub-retinal injection of 1×10^10 gp in 1 eye (MacLaren, Groppe et al. 2014). Six months after receiving AAV2-REP1, 2 patients with impaired vision at baseline gained 21 letters and 11 letters in best corrected visual acuity (BCVA) (i.e., >4 and >2 lines of vision, respectively). All other

patients who received the full dose of vector, and all of whom had near normal BCVA at baseline, did not suffer any clinically significant deterioration in vision post-treatment. From a safety perspective, the GTMP and surgical application method appeared to be generally well tolerated. A second cohort of 8 patients was treated with a single sub-retinal injection of 1×10^11 gp in 1 eye. Following favorable results, 3 other investigator-sponsored studies were then initiated in which a total of 26 adult male CHM patients received a single sub-retinal injection of 1×10^11 gp AAV2-REP1 in 1 eye . The initial results of these trials are consistent with improved rod and cone function, despite any negative effects of retinal detachment, and the gene therapy was generally well tolerated. AAV2-REP1 gene therapy has been shown to positively affect visual acuity (VA), considering both maintenance and stabilization of vision in the majority of subjects, as well as improvement of vision in subsets of CHM subjects for up to 2 years and beyond. This clinical evidence supports the durability of the therapeutic effect of AAV2-REP1 for treating CHM.

These findings lend support to further assessment of AAV2 gene therapy in the treatment of CHM. NightstaRx is currently sponsoring a multi-centre, global Phase 3 study of a single subretinal administration of AAV2-REP1 for the treatment of CHM (NSR-REP-01, the STAR study, NCT03496012).

Considering that CHM affects both eyes, exploration of bilateral AAV2-REP1 administration is desirable. As noted, emerging data from non-clinical and clinical studies with AAV2 vectors demonstrate that these vectors illicit a minimal immune response, including after bilateral administration. The aim of this study is to provide important insight into the safety and tolerability of sequential, bilateral treatment with AAV2-REP1.

5.4 Risk / Benefit Assessment

No treatment currently exists for CHM.

The non-clinical studies conducted with AAV2-REP1 showed that it provides efficient and functional transgene expression in CHM mouse and human cells, as well as in mouse and human RPE and photoreceptors, without overt toxicity. Results from the 26-week single-dose combined toxicity/biodistribution study conducted in rats indicate that administration of AAV2-REP1 by single sub-retinal injection to both eyes is well tolerated at dose levels of 1×10^9 and 6×10^9 gp/eye (equal to 1×10^11 and 6×10^11 gp/eye in humans) when evaluated 4 and 26 weeks after injection. Minor reductions in some ERG parameters, ocular inflammation, and microscopic signs of retinal/corneal degeneration were observed and were considered procedure-related or not biologically significant.

In humans, application of AAV2-REP1 to the surface of the retina requires retinal detachment via vitrectomy. The 2-step procedure employed in delivering AAV2-REP1 sub-retinally (see Section 9.4), allows the management of unexpected surgical complications of retinal detachment before the AAV2-REP1 is applied. Furthermore, since the volume of fluid required to detach the fovea is variable, by excluding the vector from the first step, and administering the vector after successful retinal detachment has been achieved, a precise, consistent dose in terms of genome particles can be applied into the sub-retinal space.

Nevertheless, sub-retinal injection of AAV2-REP1 carries the risks associated with vitrectomy and retinal detachment, which include intra-operative and post-operative complications; infection (most notably infectious endophthalmitis); low and elevated IOP; choroidal detachment; persistent retinal detachment, retinal tears, holes and breaks; macular hole and macular oedema; vitreous haemorrhage; visual impairment; metamorphopsia; and photopsia (Park, Marcus et al. 1995, Thompson, Sjaarda et al. 1996, Banker, Freeman et al.

1997, Cheng, Azen et al. 2001, Anderson, Fineman et al. 2006, Stein, Zacks et al. 2009, Recchia, Scott et al. 2010). Post-operative intraocular inflammation caused by vitrectomy is often associated with transient or sometimes permanent visual impairment. Another complication of vitrectomy is cataract formation, which may require an additional surgical procedure (cataract extraction) (Park, Marcus et al. 1995, Cheng, Azen et al. 2001, Recchia, Scott et al. 2010). In addition, the surgical procedure is performed under general anesthesia, which includes the risks of dizziness, confusion, nausea and vomiting.

Loss of VA has been observed post-treatment with AAV2-REP1. This VA loss was determined by the Investigators to be plausibly related to the administration of AAV2-REP1, however without definitively attributing the cause to either the study procedure or the GTMP. Complications from the surgical procedure are expected and are not considered reportable as suspected unexpected serious adverse reactions (SUSARs) unless also assessed to be at least possibly related to AAV2-REP1. The Reference Safety Information described in the current version of the Investigator's Brochure for AAV2-REP1 defines *visual acuity reduced* as an expected serious adverse drug reaction, with a frequency of 4/79 (5.1%), defined as common (≥1/100 to <1/10). Loss of VA is considered an identified risk of AAV2-REP1. Inflammation is a potential risk of AAV2-REP1.

See the Investigator's Brochure for further details.

Thus, although there are risks associated with the administration of the study treatment via vitrectomy and retinal detachment, the potential for benefit in the form of improved VA that may be provided to subjects with CHM following treatment with AAV2-REP1 provides an acceptable risk-benefit profile for participation in this study.

6 STUDY OBJECTIVES AND ENDPOINTS

6.1 Objectives

The objective of the study is to evaluate the safety of bilateral, sequential sub-retinal administration of a single dose of AAV2-REP1 in adult male subjects with CHM.

6.2 Endpoints

The safety of bilateral administration of AAV2-REP1 will be evaluated with the following safety measures:

- BCVA as measured by the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart
- Ophthalmic examination assessments (including intraocular pressure [IOP], slit lamp examination, lens opacity grading and dilated ophthalmoscopy)
- Spectral domain optical coherence tomography (SD-OCT)
- Fundus autofluorescence (AF)
- Fundus photography
- Microperimetry
- AE reporting
- Vector shedding post-treatment
- Immunogenicity sampling post-treatment
- Vital signs

The following will also be considered secondary efficacy endpoints:

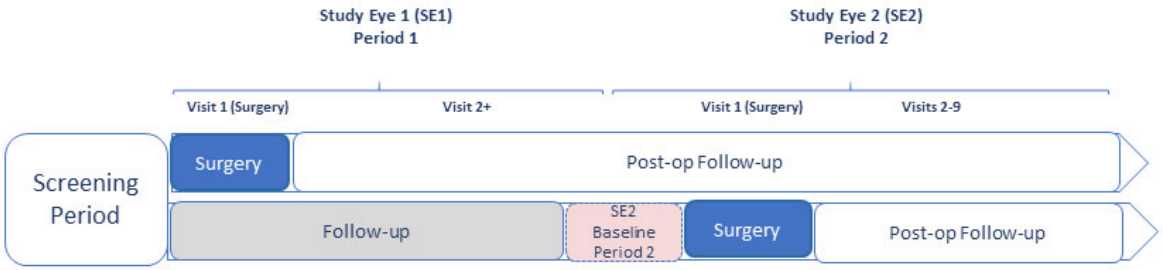
- Change from Baseline in BCVA as measured by the ETDRS chart
- Change from Baseline in AF
- Change from Baseline in SD-OCT
- Change from Baseline in microperimetry

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design

This is a multi-centre, open-label, prospective, 2-period, bilateral interventional safety study of AAV2-REP1 in adult male subjects with genetically confirmed CHM. The study will consist of a Screening Period followed by 2 treatment periods (Period 1 and Period 2) with up to 9 visits per treatment period. A second baseline visit prior to surgery of SE2 (Period 2, Visit 1) may be required. A study schematic is presented in **Figure 1**.

Figure 1 Overall Study Schematic



During the Screening Period, subjects will be assessed for eligibility of both eyes. The Investigator will assign the order in which the eyes are treated (i.e., study eye 1 [SE1]-Period 1 and study eye 2 [SE2]-Period 2, respectively) with a number assigned to each eye. This will be done in collaboration with the subject; however, the worse eye will generally be selected for first treatment. Study eye selection and eligibility for surgery will then be confirmed collectively by the Patient Eligibility Review Committee, which includes the Investigator, a consulting surgeon, and the Sponsor (see the Patient Eligibility Review Plan). The estimated target interval between the surgical procedures of SE1 and SE2 will be determined at the Screening Visit.

Each study eye will be followed for at least 12 months post-treatment, for up to 9 visits per treatment period: Visit 1 (Day 0, Injection Day Visit); Visit 2 (Day 1, post-operative follow-up visit); Visit 3 (Day 3 + 2 Days); Visit 4 (Day 7 -1/+2 Days); Visit 5 (Day 14 ± 3 Days); Visit 6 (Month 1 ± 7 Days); Visit 7 (Month 3 ± 14 Days); Visit 8 (Month 6 ±14 Days) and Visit 9 (Month 12 ± 14 Days; end of study visit).

At Period 1, Visit 1 (Day 0, Injection Day Visit for SE1), subjects will undergo a vitrectomy with retinal detachment and receive a sub-retinal injection of AAV2-REP1 in SE1. Visits 2-9 will then be conducted according to the Schedule of Study Procedures, unless Period 2 commences during the time of follow-up for SE; in which case, the study visits will continue following the schedule for SE2. Ophthalmic assessments during Period 1, Visits 2-9 will be performed on both eyes.

The interval between SE1 and SE2 treatment is expected to vary among subjects from weeks to many months. While the interval will be decided on a case-by-case basis, an effort will be made to schedule varying treatment intervals in order to better characterise the immunological and safety profile of sequential treatment administration. Thus, approximately 20 subjects each will be treated with a short- (<6 month), medium- (6-12 months), or long- (>12 month) surgery window between treatment of the first and second

eye. Because the timing of the second surgery visit (Period 2, Visit 1) will vary among subjects, the duration of study Period 1 will also be variable.

The post-operative outcome and visual function of SE1 will inform the scheduling of Period 2. Signs of post-operative inflammation or other post-operative sequelae, as judged by the Investigator, should be resolved in SE1 for the subject to continue to Period 2. After comprehensive evaluation of all safety assessments and visual functional tests of SE1 and SE2, the Investigator will determine the eligibility of the subject and decide the appropriate time to schedule SE2, Visit 1.

All baseline ophthalmic assessments must be conducted within 10 weeks prior to SE2 surgery (Period 2, Visit 1). If these assessments are not available within the required timeframe, a Baseline Visit must be added to collect current baseline data on SE2. Study eye selection and eligibility for surgery will then be confirmed collectively by the Patient Eligibility Review Committee, which includes the Investigator, a consulting surgeon, and the Sponsor (see the Patient Eligibility Review Plan). If, at that time, BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the Patient Eligibility Review Committee: 1) the subject is found eligible by all other inclusion and exclusion criteria, and 2) the subject is considered an appropriate candidate who has potentially modfiable disease.

At Period 2, Visit 1 (Day 0, Injection Day Visit for SE2) subjects will undergo a vitrectomy with retinal detachment and sub-retinal injection of AAV2-REP1 in the contralateral, untreated eye (SE2). Post-treatment, subjects will no longer follow the Period 1 visit schedule, but will instead attend Period 2, Visits 2-9 according to the Schedule of Study Procedures. Ophthalmic assessments scheduled for Period 2, Visits 2-9 will be performed on both eyes.

Study data will thus be collected for both eyes. Subjects will be assessed for safety and efficacy throughout the study, as outlined in the Schedule of Study Procedures (Table 1). The safety evaluation will be based on a full ophthalmic examination (indirect ophthalmoscopy, slit lamp examination, IOP, anterior chamber and vitreous inflammation grading and lens opacities classification system [LOCS] III cataract grading); fundus photography; AE reporting; vector shedding and immunogenicity assessment and vital signs. The efficacy evaluation will be based on BCVA (as measured by the ETDRS chart), AF, SD-OCT and microperimetry. Any safety information collected as a result of the efficacy assessments (e.g., BCVA) will also be used in the overall safety evaluation, as applicable.

Subjects who develop cataracts may undergo cataract surgery if deemed clinically necessary. If cataract surgery is performed, it should be carried out at least 4 weeks before Month 12 (Visit 9) for the respective eye. The cataract surgery timing must be discussed with the medical monitor prior to the procedure.

A subject is considered to have completed the study if he completes Period 2, Visit 9 (Month 12). The end of the trial is the date the last subject completes Period 2, Visit 9 (Month 12) assessments (or early termination [ET] assessments in the event of premature discontinuation) or the date of last data collection if the last subject is lost to follow-up. After study completion, subjects will be invited to participate in a long-term safety follow-up study that will permit continued safety and efficacy monitoring over an extended period of time post-treatment.

Subjects Previously Treated with AAV2-REP1

Subjects who have previously participated in a clinical trial of AAV2-REP1 for the treatment of CHM and have received AAV2-REP1 in one eye may be eligible for participation in this study. In these cases, only the subject's untreated eye will be assessed for eligibility per Section 8. Following the Screening Visit and determination of eligibility, the subject will move directly into Period 2 for treatment of SE2.

For subjects who received previous treatment with AAV2-REP1, biological samples must be available to obtain an adequate immunology profile related to treatment of the first eye in the previous study. These subjects will require Sponsor approval before being enrolled into the study.

7.2 Discussion of Design

Since CHM is a bilateral disease, this open-label study is being conducted to assess the safety of AAV2-REP1 administered to both eyes of subjects with CHM. All enrolled subjects will receive treatment with AAV2-REP1 at a dose of 1×10^11 AAV2-REP1 gp/eye. Bilateral sub-retinal administration of AAV2-REP1 was explored in a pre-clinical toxicology study in rats and was found to be well tolerated and not associated with drug-related AEs. Furthermore, sequential sub-retinal administration of an AAV2 vector to the contralateral eye in children and adults with Leber's congenital amaurosis was demonstrated to be safe and efficacious, irrespective of baseline immune status (Bennett, Wellman et al. 2016).

The planned sample size (approximately 60 subjects, with a total of 120 treated eyes) is considered sufficient to characterise the immunological, vector shedding, and safety profiles of bilateral retinal gene therapy with AAV2-REP1. A prospective trial period of at least 12 months following the administration of AAV2-REP1 in each eye is considered a sufficient period of time to observe any treatment related safety concerns.

The dose of vector being employed in this study is based on previous clinical trials using the AAV2 vector with a chicken β actin (CBA) promoter (Maguire, High et al. 2009, MacLaren, Groppe et al. 2014), and the Phase 1 / 2 investigator-sponsored clinical studies in which AAV2-REP1 was administered to subjects with CHM (MacLaren, Groppe et al. 2014) (Dimopolous, Hoang et al. 2018, Fischer, Ochakovski et al. 2018, Lam, Davis et al. 2019).

Application of AAV2-REP1 to the retina requires retinal detachment following vitrectomy. The risks of vitrectomy and retinal detachment are summarized in Section 5.4.

In line with guidance from regulatory agencies, the treatment intervals were chosen to comprehensively characterise the immunological profile following bilateral, sequential AAV2-REP1 treatment, and to reflect real-world application of gene therapy. As noted, both preclinical data with AAV2-REP1, and clinical data with sub-retinal administration of an AAV2 vector in subjects with Leber's congenital amaurosis, support bilateral administration of AAV2-REP1 in humans.

To minimise inflammation resulting from surgery, and potential or unexpected immune responses to vector/transgene, all subjects will be given a course of oral prednisone/prednisolone, initiated 2 days prior to surgery on both SE1 and SE2. The regimen has been modified from the 17-day protocol established in the Philadelphia AAV gene therapy clinical trial (Maguire, Simonelli et al. 2008), with the allowance of an extra 4 days for tapering the dose at the end of the course. The corticosteroid regimen is 1 mg/kg/day (for a maximum of 80 mg/daily) prednisone/prednisolone for a total of 10 days (beginning 2 days before the vector injection, on the day of injection, and then for 7 days); followed by

0.5 mg/kg/day for 7 days; 0.25 mg/kg/day for 2 days; and 0.125 mg/kg/day for 2 days (21 days in total). See Section 9.8 for details.

8 SELECTION AND WITHDRAWAL OF SUBJECTS

The study will enroll approximately 60 subjects with CHM, for a total of 120 treated eyes (see Section 13.1 for discussion of the sample size determination).

8.1 Inclusion Criteria

During the Screening Period, subjects will be found eligible for study participation if they meet all of the following inclusion criteria.

1. Are willing and able to give informed consent for participation in the study to have both eyes treated
2. Are male and ≥18 years of age
3. Have documentation of a genetically-confirmed diagnosis of CHM
4. Have active disease clinically visible within the macular region of both eyes
5. Have a BCVA of ≥34 ETDRS letters (20/200 or better Snellen acuity) in both eyes, or in the untreated eye, if the other eye was previously treated with AAV2-REP1*

   *If previously treated with AAV2-REP1 in an antecedent study, subjects may be eligible for participation following Sponsor approval.
6. For subjects who received treatment with AAV2-REP1 in an antecedent study, have biological samples available to complete an adequate immunology profile

8.2 Exclusion Criteria

During the Screening Period, subjects will be found not eligible for study participation if they meet any of the following exclusion criteria.

1. Have a history of amblyopia or inflammatory disorder in either eye
2. Are unwilling to use barrier contraception methods or abstain from sexual intercourse for a period of 3 months following treatment with AAV2-REP1 in either eye
3. Have had previous intraocular surgery performed within 3 months of the Screening Visit in either eye
4. Have any other significant ocular or non-ocular disease/disorder which, in the opinion of the Patient Eligibility Review Committee or Investigator, may either put the subjects at risk because of participation in the study, or may influence the results of the study or the subject's ability to participate in the study. This includes but is not limited to a potential subject:
   - with a contraindication to oral corticosteroid (e.g., prednisolone/prednisone)
   - with clinically significant cataract in either eye
   - who, in the clinical opinion of the Patient Eligibility Review Committee or Investigator, is not an appropriate candidate for sub-retinal surgery (see the Patient Eligibility Review Plan)
5. Have participated in another research study involving an investigational product in the past 12 weeks or received a gene/cell-based therapy at any time previously, except if treated within an antecedent study with AAV2-REP1

8.3 Subject Withdrawal Criteria

Each subject has the right to withdraw from the study at any time without prejudice. In addition, the Investigator may discontinue a subject from the study at any time if the Investigator considers it necessary for any reason, including:

- Significant protocol deviation
- Significant non-compliance with study requirements
- AE which results in an inability to continue to comply with study assessments
- Lost to follow up
- Other (to be specified on the electronic case report form [eCRF]).
    - If severe ocular inflammation (e.g. endophthalmitis) or any other post-operative sequela that is unresponsive to treatment occurs in SE1, the second eye should not be treated. However, Period 1 should be continued for monitoring of safety for the duration of the study.
    - At the discretion of the Investigator, if a persistent post-operative complication or poor visual function outcome presents post-operatively in SE1, the second eye may not be treated. However, Period 1 should be continued for monitoring of safety for the duration of the study.

In the event that a subject discontinues the study, the reason for withdrawal is to be recorded in the eCRF, and the site should use every reasonable effort to ensure that an end of trial (ET) Visit is conducted as outlined in the Schedule of Study Procedures (Table 1). If the subject is withdrawn due to an AE, the Investigator will arrange for follow-up until the event has resolved, subsided, stabilised, or the subject withdraws consent or is lost to follow-up. For subjects who withdraw consent, data will be collected through their last available study visit. Subjects withdrawn from the study may possibly be replaced. Subjects who withdraw from the study must undergo the ET Visit assessments. See Table 1.

Withdrawal from the study will not result in the exclusion of a subject's data acquired up to the point of withdrawal.

9 STUDY TREATMENT

9.1 Treatments Administered

Eligible subjects will undergo vitrectomy and retinal detachment in each eye. At Period 1, Visit 1 (Day 0, the Injection Day Visit for SE1), subjects will receive in SE1 a sub-retinal injection of up to 0.1 mL of study drug containing 1×10^11 AAV2-REP1 gp. At Period 2, Visit 1 (Day 0, the Injection Day Visit for SE2), subjects will receive in SE2 up to 0.1 mL of AAV2-REP1 via sub-retinal injection.

9.2 Description of Study Drug

The AAV2 vector contains recombinant human cDNA encoding REP1 (AAV2-REP1). The vector genome (AAV2-CBA-hREP1-WPRE-BGH) is comprised of a strong constitutive expression cassette, a hybrid CBA promoter, the human cDNA encoding REP1, a modified woodchuck hepatitis post-transcriptional regulatory element (WPRE) sequence, and a bovine growth hormone polyadenylation (BGH-polyA) sequence flanked by AAV2 inverted terminal repeats. The cDNA fragment was originally isolated from a human retinal cDNA library from unaffected individuals.

The AAV2-REP1 drug product is formulated in a sterile, 20 mM Tris-buffered solution, pH 8.0, and contains 1 mM $MgCl_2$, 200 mM NaCl and 0.001% PF68. The drug product is a clear to slightly opalescent, colourless, sterile-filtered suspension with a target concentration of 1×10^12 gp/mL.

9.3 Packaging, Labeling, and Storage

AAV2-REP1 is currently supplied in sterile single-use vials, stoppered and capped. A total of 0.3 mL vector suspension will be supplied for each eye to be treated. Prior to shipment, each vial will be placed in a labeled secondary container. The drug product is to be stored at <−60°C (<−76°F) in a controlled access, temperature monitored freezer.

The GTMP will be labeled in compliance with regulatory standards.

9.4 Vitrectomy Procedure and Injection of AAV2-REP1

Injection of AAV2-REP1 is to be performed by an appropriately qualified and experienced retinal surgeon. All surgeons must have completed surgical training and obtained certification by NightstaRx to perform the study procedure, before treating a study participant. Training conducted as part of a previous AAV2-REP1 clinical trial or alternative AAV NightstaRx-sponsored gene therapy trial, can be used as the basis for approval.

Due to the complexity and unpredictability of detaching the retina in CHM, in which the retina and choroid can be extremely thin and fused in places, a modification to the technique of sub-retinal gene therapy has been developed. This involves performing the vector delivery in 2 steps after vitrectomy. An advantage of a 2-step procedure is that any unexpected complications of retinal detachment can be managed conservatively, minimising concerns about the vector escaping into the vitreous. Further, the injection could be deferred until a later date if, for instance, a macular hole was created which required treatment with gas. Also, since the volume of fluid required to detach the fovea is variable, by removing the vector from the first step, a precise consistent dose in terms of genome particles can still be applied into the sub-retinal space.

Initially, subjects will undergo a standard vitrectomy and detachment of the posterior hyaloid in the respective study eye (Figure 2). All surgeries will be conducted using the standard BIOM vitrectomy system. A 23-gauge sutured approach is usually favored to avoid any potential risks of wound leakage. The retina will be detached with 0.1-0.5 mL of balanced salt solution (BSS) injected through a 41-gauge sub-retinal cannula connected to a vitreous injection set.

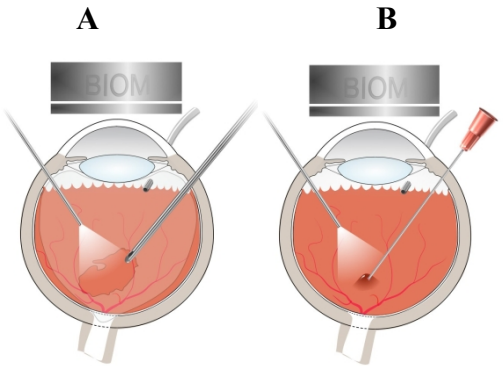
In the second step of the procedure, the BSS cannula is removed from the eye and AAV2-REP1 is prepared for injection. A dose of up to 1.0×10^11 gp of AAV2-REP1 is injected into the sub-retinal space through the same entry site. To improve visualization of the vector and facilitate dosing, surgeons are given the option of adding a minute quantity of trypan blue ophthalmic solution (~6 µL) to the vector solution. See the GEMINI Surgical Manual for further details.

Prior to administration, the vector needs to be primed in the 1-mL syringe to avoid formation of air bubbles, and a connector is used so that the 1-mL syringe can be connected to the constant pressure line of the vitrectomy machine. The sub-retinal injection will target any area of the macula, but also include the fovea, if possible. In each case, the vector is injected so that the sub-retinal fluid overlies all edge boundaries of the central region that has yet to undergo chorioretinal degeneration, as identified by fundus AF. After wound closure, care will be taken to dispose of all irrigating fluids that may have passed through the eye to limit potential vector spread. See the GEMINI Surgical Manual for further details.

Subjects will be carefully monitored for the occurrence of AEs peri- and post-operatively. All AEs, irrespective of relationship to the study drug and/or the surgical procedure, will be captured in the subject's medical record and reported in the eCRF.

Air fluid exchange during pars plana vitrectomy is not required for this surgical procedure. However, if an air fluid exchange is considered necessary, patients must be instructed to avoid air travel, travelling to high elevations or scuba diving until the air bubble has completely dissipated from the eye. It may take one week or more following injection for the air bubble to dissipate. A change in altitude while the air bubble is still present can result in irreversible vision loss. The dissipation of the air bubble should be verified through meticulous ophthalmic examination.

Figure 2 Vitrectomy and Sub-Retinal Injection of AAV2 Vector



(A) A standard vitrectomy through the BIOM operating system to remove the vitreous gel is followed by (B) a 2- step procedure: 1) retinal detachment by injection of BSS; 2) injection of a volume of up to 0.1 mL vector suspension through a 41-gauge cannula into the sub-retinal space.

9.5 Randomisation

Not applicable for this study.

9.6 Study Masking

Not applicable for this study.

9.7 Study Drug Accountability

Records of the receipt and dispensing of study drug will be kept by each study centre until the end of the study to provide complete accounting of all used and unused study drug. Dispensation logs will be checked by the Sponsor (or its designee). Study centres will destroy all used vials in accordance with local regulations, and will return all unused study drug to the Sponsor (or its designee) at the end of the study. Final drug accountability will be verified by the Sponsor (or its designee).

9.8 Concomitant Therapy

Subjects cannot have participated in another research study involving an investigational product in the past 12 weeks or received a gene/cell-based therapy at any time previously, except if treated within another study with AAV2-REP1.

Throughout the study, subjects may be prescribed any concomitant medications, procedures, or treatments deemed necessary to provide adequate supportive care. Details of concomitant medications, procedures and/or treatments will be collected during the Screening Period and updated at every study visit (including the ET Visit, if applicable). Concomitant medications (including oral corticosteroid), procedures and/or treatments taken during the study are to be recorded in the subject's medical records and eCRF; an exception to this is any medication used in the course of conducting study assessments (e.g., ophthalmic dyes, topical anaesthesia, dilating eye drops).

In addition, all subjects will be prescribed a course of oral corticosteroids.

The oral prednisone / prednisolone regimen has been modified from the 17-day protocol established in the Philadelphia voretigene neparvovec-rzyl gene therapy clinical trial for treatment of patients with confirmed bi-allelic RPE65 mutation-associated retinal dystrophy (Maguire, Simonelli et al. 2008), with the allowance of an extra 4 days for tapering the dose at the end of the course. The corticosteroid regimen is 1 mg/kg/day prednisone/prednisolone (not to exceed 80 mg/daily) for a total of 10 days (beginning 2 days before the vector injection, on the day of injection, and then for 7 days); followed by 0.5 mg/kg/day for 7 days; 0.25 mg/kg/day for 2 days; and 0.125 mg/kg/day for 2 days (21 days in total; Study Day (SD) -2 through SD18). This regimen is outlined in detail below.

Full Corticosteroid Regimen

Prednisone or prednisolone administered as follows:

- SD -2 through SD 7 (10 days): 1 mg/kg by mouth daily (not to exceed 80 mg/daily)
- SD 8 through SD 14 (7 days): 0.5 mg/kg/day by mouth daily
- SD 15 through SD 16 (2 days): 0.25 mg/kg/day by mouth daily
- SD 17 through SD 18 (2 days): 0.125 mg/kg/day by mouth daily

For SE1, the Full Corticosteroid Regimen should be initiated as described.

For SE2 surgeries that occur on day 21 or later, the Full Corticosteroid Regimen should be initiated as described, beginning 2 days before the scheduled SE2 surgery.

If SE2 is to be performed prior to Study Day 21, then the Full Corticosteroid Regimen should be initiated 2 days prior to the scheduled surgery for SE2, and this will supersede the steroid taper in progress for SE1. The full 21 days of treatment will then be completed for SE2.

Details of corticosteroid usage will be captured by each subject in a diary card.

If inflammation is observed in the study eye, and in the opinion of the Investigator additional treatment with corticosteroid medication is indicated, corticosteroid therapy may be increased during the taper period (to a maximum of 1 mg/kg/day), may be reinitiated following completion of the taper, and/or may be supplemented by intraocular corticosteroids.

9.9 Treatment Compliance

This study involves a single sub-retinal injection of a volume of up to 0.1 mL AAV2-REP1 in each eye. Measure of treatment compliance with AAV2-REP1 is therefore not necessary.

Compliance with the use of prednisone/prednisolone will be captured in the eCRF; subject diary cards will be provided to participants prior to each surgery.

10 STUDY VISITS AND PROCEDURES

The schedule of study procedures is presented in Table 1.

At each study visit, an attempt should be made to perform all procedures in both eyes.

10.1 Screening Period

The Investigator will explain the study purpose, procedures and subject responsibilities to each potential study subject. The subject's willingness and ability to meet the protocol requirements will be determined.

Prior to any study-specific procedure, written informed consent will be obtained. The subject will sign and date 1 copy of the consent form in the presence of the Investigator or his/her designee. The original signed form will be retained at the study site and an additional copy will remain in the subject's medical records; a copy will also be given to the subject.

After informed consent has been obtained, the subject will be allocated a subject identifier and evaluated to determine study eligibility.

Screening procedures will consist of the following (all ophthalmic assessments will be conducted on both eyes):

- Demography, medical and ocular history
- Vital signs
- Weight
- Vector shedding sampling – blood, tears (both eyes), urine, saliva
- Immunogenicity sampling
- BCVA
- Microperimetry
- Full ophthalmic examination, including IOP, a slit-lamp examination, lens opacity, and dilated fundus examination
- SD-OCT
- AF
- 7-field colour fundus photography (including stereo photographs for fields 1, 2, and 3)
- AE and serious adverse event (SAE) monitoring
- Concomitant medication, procedures, and treatment review

Subjects who meet all of the inclusion criteria and none of the exclusion criteria will be assigned a subject number and will be enrolled into the study. The Investigator will assign the order in which the eyes are treated (i.e., SE1 and SE2, respectively). This will be done in collaboration with the subject; however, the worse eye will generally be selected for treatment first. Study eye selection and eligibility for surgery will then be confirmed collectively by the Patient Eligibility Review Committee, which includes the Investigator, a consulting surgeon, and the Sponsor (see the Patient Eligibility Review Plan). The target interval between the surgical procedures of SE1 and SE2 will be determined at this visit.

For each subject, the interval between SE1 and SE2 treatment is expected to range from weeks to months. While this interval will be decided on a case-by-case basis, an effort will

be made to schedule varying treatment intervals in order to better characterise the immunological and safety profile of sequential treatment administration and to reflect the anticipated real-world application of the study drug. Thus, approximately 20 subjects will be treated with a short- (<6 month), medium- (6-12 months), or long- (>12 month) duration surgery window between treatment of the first and second eye. Because the timing of the second surgery visit (Period 2, Visit 1) will vary among subjects, the duration of study Period 1 will also be variable. To avoid unnecessary study visits, every effort should be made by the Investigator to schedule Period 2, Visit 1 to coincide with a planned Period 1 post-treatment visit.

The next study visit (i.e., surgery and dosing, Period 1, Visit 1) is to be scheduled within 10 weeks of the Screening Period. Subjects will attend Period 1, Visits 2-9 unless the second surgery visit (Period 2, Visit 1) begins, which will mark the start of study Period 2. Because the timing of the second surgery visit (Period 2, Visit 1) will vary among subjects, the duration of study Period 1 will also be variable.

Subjects will be treated with oral corticosteroids (e.g., prednisolone / prednisone) during the peri-operative period for each eye. They will be instructed to start taking the drug 2 days before their surgery at Period 1, Visit 1 for SE1. For SE1, the Full Corticosteroid Regimen should be initiated, as described in Section 9.8.

For SE2 surgeries that occur on Day 21 or later, the Full Corticosteroid Regimen should be initiated as described, begining 2 days before the scheduled surgery.

If SE2 is to be performed prior to Study Day 21, then the Full Corticosteroid Regimen should be initiated 2 days prior to the scheduled surgery for SE2, and this will supersede the steroid taper in progress for SE1. The full 21 days of treatment will then be completed for SE2.

Subjects will be issued a diary card to capture corticosteroid compliance. Subjects will also be instructed to use barrier contraception or abstain from sexual intercourse for a period of 3 months from the time they are treated.

The schedule of procedures is identical for Periods 1 and 2; during each period, procedures are to be conducted in both SE1 and SE2.

If a subject has previously participated in a clinical trial of AAV2-REP1 for the treatment of CHM and has received AAV2-REP1 in one eye, the untreated eye will be assigned as SE2 at the Screening Visit and the subject will immediately enter Period 2.

10.1.1 Baseline Visit, Period 2 (<10 Weeks from Visit 1, Period 2 [SE2])

The following baseline assessments for Period 2 must be performed within 10 weeks prior to surgery for SE2 (Visit 1, Period 2). If the assessments have not been performed within a regularly scheduled visit within this window, an additional visit is required.

- Vital signs
- Weight
- Vector shedding sampling – blood, tears (both eyes), urine, saliva
- Immunogenicity sampling
- BCVA
- Microperimetry

- Full ophthalmic examination, including IOP, a slit-lamp examination, lens opacity, and dilated fundus examination
- SD-OCT
- AF
- 7-field colour fundus photography (including stereo photographs for fields 1, 2, and 3)
- AE and SAE monitoring
- Concomitant medication, procedures and treatment review

Eligibility for SE2 surgery will then be confirmed collectively by the Patient Eligibility Review Committee, which includes the Investigator, a consulting surgeon, and the Sponsor (see the Patient Eligibility Review Plan).

If BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the Patient Eligibility Review Committee:

1. the subject is found eligible by all other inclusion and exclusion criteria, and
2. the subject is considered an appropriate candidate who has potentially modifiable disease.

10.2 Period 1 and 2, Visit 1 (Day 0, Injection Day Visit)

At Visit 1 (Day 0, Injection Day Visit), the following assessments will be performed:

- Full ophthalmic examination, including IOP, a slit-lamp examination, lens opacity, and dilated fundus examination
- AE/SAE monitoring
- Concomitant medication, procedures, and treatment review (including review of the corticosteroid diary card)

It may be necessary for subjects to undergo a pre-surgical workup according to local hospital procedures (e.g., blood draw for anesthetist) which are outside of the protocol-defined assessments. Any assessments performed outside of the protocol will not be collected as part of the study analysis.

Subjects will then undergo vitrectomy and receive a volume of up to 0.1 mL sub-retinal injection of AAV2-REP1, containing 1×10^11 gp (see Section 9.4 for details). Subjects will be carefully monitored for the occurrence of AEs during the procedure. Subjects will return to the site 1, 3, 7 and 14 days after surgery for post-operative follow-up (Visit 2 [Day 1], Visit 3 [Day 3 + 2 Days], Visit 4 [Day 7 -1/+2 Days] and Visit 5 [Day 14 ± 3 Days], respectively).

The post-operative outcome and visual function of SE1 will inform the scheduling of Period 2. Significant signs of post-operative inflammation or other post-operative sequelae, as judged by the Investigator, should be resolved in SE1 for the subject to continue to Period 2. After a comprehensive evaluation of all safety assessments and visual functional tests of SE1 and SE2, the Investigator will decide the appropriate time to schedule SE2, Visit 1. If severe ocular inflammation or any other post-operative sequela that is unresponsive to treatment occurs in SE1, the second eye should not be treated. However, Period 1 should be continued for monitoring of safety for the duration of the study.

To allow accurate characterisation of AAV2-REP1 safety and immunogenicity profile, treatment of SE2 should not occur if a previous intraocular surgery was performed on the same eye within 3 months of the planned treatment date. If intraocular surgery has occurred within 3 months, treatment of SE2 should be delayed until such a time that the 3-month interval has elapsed and there is complete post-operative recovery of the eye.

10.3 Period 1 and 2, Visit 2 (Post-operative Day 1)

For Visit 2, ocular assessments and procedures will be performed on each eye. At Visit 2 (Post-operative Day 1), subjects will return to the site for their first post-operative visit for the same eye that underwent surgery at Visit 1 (Period 1 [SE1] or Period 2 [SE2]). The following assessments will be performed:

- Vital signs
- Vector shedding sampling – blood, tears (both eyes), urine, saliva
- Immunogenicity sampling
- BCVA
- Full ophthalmic examination, including IOP, a slit-lamp examination, lens opacity, and dilated fundus examination
- SD-OCT
- AE/SAE monitoring
- Concomitant medication, procedures, and treatment review (including review of the corticosteroid diary card)

10.4 Period 1 and 2, Visit 3 (Post-operative Day 3 + 2 Days)

For Visit 3 (Post-operative Day 3 + 2 Days), ocular assessments and procedures will be performed on each eye. At Visit 3 (Day 3+ 2 Days), subjects will return to the site for their second post-operative visit for the same eye that underwent surgery at Visit 1 (Period 1 [SE1] or Period 2 [SE2]). The following assessments will be performed:

- Vital signs
- Vector shedding sampling – blood, tears (both eyes), urine, saliva
- BCVA
- Full ophthalmic examination, including IOP, a slit-lamp examination, lens opacity, and dilated fundus examination
- AE/SAE monitoring
- Concomitant medication, procedures and treatment review (including review of the corticosteroid diary card)

10.5 Period 1 and 2, Visit 4 (Post-operative Day 7 -1/+2 Days)

For Visit 4 (Post-operative Day 7 -1/+2 days), ocular assessments and procedures will be performed on each eye. At Visit 4 (Day 7 -1/+2 days), subjects will return to the site for their third post-operative visit for the same eye that underwent surgery at Visit 1 (Period 1 [SE1] or Period 2 [SE2]). The following assessments will be performed:

- Vector shedding sampling – blood, tears (both eyes), urine, saliva

- Immunogenicity sampling
- BCVA
- Full ophthalmic examination, including IOP, a slit-lamp examination, lens opacity, and dilated fundus examination
- SD-OCT
- AF
- AE/SAE monitoring
- Concomitant medication, procedures and treatment review (including review of the corticosteroid diary card)

10.6 Period 1 and 2, Visit 5 (Post-operative Day 14 ± 3)

For Visit 5 (Post-operative Day 14 ± 3 days), ocular assessments and procedures will be performed on each eye. At Visit 5 (Day 14 ± 3 days), subjects will return to the site for their fourth post-operative visit for the same eye that underwent surgery at Visit 1 (Period 1 [SE1] or Period 2 [SE2]). The following assessments will be performed:

- Vector shedding sampling – blood, tears (both eyes), urine, saliva
- Immunogenicity sampling
- BCVA
- Full ophthalmic examination, including IOP, a slit-lamp examination, lens opacity, and dilated fundus examination
- SD-OCT
- AF
- AE/SAE monitoring
- Concomitant medication, procedures and treatment review (including review of the corticosteroid diary card)

10.7 Period 1 and 2, Visit 6 (Month 1 ± 7 Days) and Visit 7 (Month 3 ± 14 Days)

For Visit 6 (Month 1 ± 7 days) and Visit 7 (Month 3 ± 14 days), ocular assessments and procedures will be performed on each eye. The following assessments will be performed:

- Vector shedding sampling – blood, tears (both eyes), urine, saliva
- Immunogenicity sampling
- BCVA
- Full ophthalmic examination, including IOP, a slit-lamp examination, lens opacity, and dilated fundus examination
- SD-OCT
- AF
- Microperimetry
- AE/SAE monitoring

- Concomitant medication, procedures and treatment review (including review/return of the corticosteroid diary card at Visit 6)

10.8 Period 1 and 2, Visit 8 (Month 6 ± 14 Days) and Visit 9 (Month 12 ± 14 Days)*

For Visit 8 (Month 6 ± 14 days) and Visit 9 (Month 12 ± 14 days), ocular assessments and procedures will be performed on each eye. The following assessments will be performed:

- Vital signs (Visit 9, Month 12 only)
- Immunogenicity sampling
- BCVA
- Full ophthalmic examination, including IOP, a slit-lamp examination, lens opacity, and dilated fundus examination
- SD-OCT
- AF
- Microperimetry
- 7-field colour fundus photography (including stereo photographs for fields 1, 2, and 3) (Visit 9, Month 12 only)
- AE/SAE monitoring
- Concomitant medication, procedures and treatment review

10.9 Early Termination (ET Visit)

In the event that a subject discontinues the study at any time, the site should use every reasonable effort to ensure that an ET Visit is conducted. The following assessments should be performed (all ophthalmic assessments will be conducted on both eyes):

- Vital signs
- Vector shedding sampling – blood, tears (both eyes), urine, saliva (only if the ET Visit occurs within 3 months post-treatment)
- Immunogenicity sampling
- BCVA
- Full ophthalmic examination, including IOP, a slit-lamp examination, lens opacity, and dilated fundus examination
- SD-OCT
- AF
- Microperimetry
- 7-field colour fundus photography (including stereo photographs for fields 1, 2, and 3)
- AE/SAE monitoring
- Concomitant medication, procedures and treatment review (including review/ return of the corticosteroid diary card, if applicable)

10.10 Unscheduled Visits

If clinically indicated, subjects may need to return to the site for an unscheduled visit. At a minimum, the following assessments will be performed (ophthalmic assessments will be conducted on any eye deemed appropriate by the Investigator):

- BCVA
- Full ophthalmic examination, including IOP, a slit-lamp examination, lens opacity, and dilated fundus examination
- SD-OCT
- AE/SAE monitoring
- Concomitant medication, procedures and treatment review (including review / return of the corticosteroid diary card, if applicable)

11 ASSESSMENT OF SAFETY

11.1 Evaluation, Recording, and Reporting Adverse Events

11.1.1 Definitions

11.1.1.1 Adverse Event

An AE is any untoward medical occurrence in a clinical investigation subject, which does not necessarily have a causal relationship with the study drug/surgical procedure. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study drug/surgical procedure, whether or not related to the investigational product or with the surgical procedure described in this protocol.

AEs are to also include any pre-existing condition (other than CHM) or illness that worsens during the study (i.e., increases in frequency or intensity).

Worsening of the signs, symptoms and visual function related to CHM is not to be considered an AE unless, in the opinion of the Investigator, the changes are not consistent with the natural progression of the disease.

11.1.1.2 Serious Adverse Event

An SAE is defined as 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/incapacity
- Is a congenital anomaly/birth defect
- Results in vision loss or is vision threatening
- Is another important medical event(s)

The term ‘life-threatening’ in the definition of ‘serious’ refers to an event in which the subject is at risk of death at the time of the event. It does not refer to an event that hypothetically might cause death if it were more severe.

Hospitalisation that was pre-scheduled prior to obtaining the consent to participate in the study or for an elective procedure or routinely scheduled treatment for a pre-existing condition which has not worsened, does not constitute an SAE.

A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the subject is hospitalized. The study site must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the subject’s consent to participate in the study.
- The condition requiring the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the subject’s consent to participate in the study and the time of the procedure or treatment.

- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission.

If a subject is hospitalized due to local requirements for administration of the study treatment, the hospitalization should not be considered an SAE unless one of the requirements in Section 11.1.1.2 is met.

Other events that may not result in death, are not life-threatening or do not require hospitalisation, may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardise the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed above.

Vision Loss to be Reported as Serious Adverse Events

Vision Loss not to be Reported as an SAE:

- Surgery-related BCVA decrease of ≥15 letters on ETDRS chart occurring within 1 day of surgery, but recovering / resolving at post-operative Days 7 and 14.

Vision Loss or Vision-Threatening Event to be Reported as an SAE:

- Surgery-related BCVA decrease of ≥15 letters on ETDRS chart that occurs within 1 day of surgery and that has not recovered* by the 1-Month Visit.
- A decrease in BCVA of ≥15 letters on ETDRS chart that occurs within 1 day of surgery, however, in the Investigator's opinion:
    - Has an evolution not consistent with the expected post-operative course;
    - May be attributable to a complication that occurred during surgery, or another untoward event, or the study drug;
    - Actually or potentially requires any surgical or medical intervention to prevent permanent loss of vision.
- Non-surgery-related, sustained (>48 hours duration) decrease from baseline in BCVA of ≥15 letters on ETDRS chart.

*Recovery / Resolution of BCVA is defined as a return to baseline BCVA within 5 letters on the ETDRS chart.

11.1.2 Recording of Adverse Events

AEs/SAEs will be collected from the time the subject provides written informed consent (Screening Visit) through Period 2, Visit 9 (Month 12 ± 14 Days or ET Visit, if applicable).

Subjects will be questioned on the occurrence of an AE at every visit including any unscheduled visit, by using non-leading questioning such as 'How have you been since the last visit?'

All AEs occurring during the study observed by the Investigator or reported by the subject, whether or not attributed to study drug or the surgical procedure, will be recorded in the subject's medical records and in the eCRF. Any clinically significant changes in laboratory results or vital sign measurements (as determined by the Investigator) are to be recorded as an AE.

Ocular AEs are to be recorded on a per-eye basis.

The following information will be recorded in the eCRF for each AE: description, date of onset and end date, outcome, severity, assessment of relatedness to study drug/study procedure, the action taken and confirmation of whether the event is considered serious (see Section 11.1.1.2 for the definition of seriousness). Follow-up information should be provided as necessary (see Section 11.1.3 for specifics on follow-up procedures).

AE severity and relationship to the study drug or the surgical procedure will be assessed at the site by the Investigator or a medically qualified designee.

AE Severity

The severity of events will be assessed on the following scale:

1 = mild (awareness of sign or symptom, but easily tolerated)

2 = moderate (discomfort sufficient to cause interference with normal activities)

3 = severe (incapacitating, with inability to perform normal activities).

AE Relationship

When assigning relatedness of the AE, consideration will be given to whether there is a plausible relationship to either the study drug or the surgical procedure. The following are definitions of relatedness that will be used in this study:

Unrelated:	The event is not reasonably related in time to the administration of the study drug/surgical procedure, or exposure of the study drug/surgical procedure has not yet occurred, or biologic plausibility does not exist
Related:	A reasonable possibility exists that the study drug / study procedure caused the AE. A suspected AE can be further defined by: *Possibly related:* a relationship is clinically or biologically reasonable relative to the administration of the study drug/surgical procedure, but the event could have been due to another equally likely cause *Probably related:* a relationship is clinically/biologically reasonable relative to the administration of the study drug/surgical procedure, and the event is more likely explained by exposure to/administration of the study drug/surgical procedure than by other factors and causes *Definitely related:* there is a causal relationship of the onset of the event, relative to administration of the study drug/surgical procedure and there is no other cause to explain the event.

When a relationship is determined to exist, the Investigator or medical designee will further define if that relationship is to the *study drug*, the *study procedure*, *both*, or *unknown*.

11.1.3 Follow-up of Adverse Events

AEs will be followed until the subject has recovered or the subject's participation in the study is complete.

Subjects who are withdrawn from the study as a result of a drug-related AE will be followed up until the event has resolved, subsided, stabilised, or the subject withdraws consent or is lost to follow-up.

All SAEs, regardless of attribution to study drug or the surgical procedure, should be followed-up until the event has resolved, subsided, stabilised, or the subject withdraws consent or is lost to follow-up. The Sponsor (or designee) will follow up on SAE reports to completion. Investigators are expected to provide the requested additional information for a complete assessment and documentation of the SAE reports in a timely manner.

11.1.4 Reporting of Serious Adverse Events

The Investigator shall immediately (within 24 hours of learning of the event) report any SAE to the Sponsor (or its designee) by completing and emailing the SAE form. For reporting purposes, the date of SAE form submission by the Investigator to the Sponsor will be designated as Day 0.. The initial report shall be promptly followed up with a more detailed report providing specifics about the subject and the event. Copies of hospital reports, autopsy reports, and other documents should be provided (if applicable).

The Sponsor will report SAEs and SUSARs to investigative sites, the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), and regulatory authorities in compliance with current regulations. All cases that are fatal or life-threatening will be reported immediately after the Sponsor received the initial report from the Investigator. All non-fatal or non-life-threatening cases will be reported within a maximum of 15 days after the initial Investigator's report. The Sponsor will also provide periodic safety reports to IRBs/IECs and regulatory authorities, as applicable. Follow-up SAE reports will be submitted within 15 days of receiving the information.

A sample SAE form is provided in the Study Operations Manual, along with the SAE reporting contact information.

11.1.5 Data Monitoring Committee

An independent Data Monitoring Committee (DMC) will be used in this study to safeguard the safety and interests of study subjects and assess the safety and risk/benefit of the gene therapy intervention during the trial.

At regular intervals during the study, the DMC will review the progress and accrued study data and provide advice to the Sponsor on the efficacy and safety aspects of the study. The DMC will also inform the Sponsor if there is a consensus that the ongoing data show that the gene therapy, its method of administration, and/or the study design are no longer in the best interests of study subjects. Details of DMC organisation and responsibilities are included within a DMC charter.

11.2 Pregnancy

Any pregnancy that occurs during the clinical study in a female partner of a study subject should be recorded on a Pregnancy Notification Form. Consent from the pregnant partner is required prior to the collection of personal data. However, the Investigator shall immediately (within 24 hours of learning of the event) report the pregnancy, with at least preliminary data, to the Sponsor (or its designee) by completing and emailing the Pregnancy Notification Form. Follow-up information including delivery, termination, and any congenital abnormality should be reported within 24 hours of the study site staff becoming aware.

11.3 Stopping Criteria

- Severe ocular inflammation (e.g. endophthalmitis) that is unresponsive to treatment should result in the affected subject not being treated in the second eye. However, the subject will continue to be followed for safety for the duration of the study.
- At the request of the DMC, the trial may be stopped based on specific safety concerns (i.e., SUSARs), not defined in the Investigator's Brochure (Section 6.3).
- The study may be discontinued if the Sponsor deems it necessary for medical, safety, regulatory, business, or other reasons consistent with applicable laws or regulations.

11.4 Laboratory Assessments

11.4.1 Vector Shedding

Blood, tears (both eyes), urine and saliva samples will be collected at the times indicated in Table 1 and tested using an appropriate assay for evidence of vector shedding and dispersion. Samples will be sent to a central laboratory for analysis. Refer to the Study Operations Manual for details on the shipping and handling of samples.

11.4.2 Immunogenicity

For the evaluation of immunogenicity, blood will be collected at the times indicated in Table 1. Selected samples will be analysed.

Immunoassays are planned to assess cell-based and antibody-based immune responses against AAV2-REP1. Enzyme-linked immunospot (ELISPOT) assays will be used for the assessment of T-cell-mediated immune responses to transgene product (REP1) and the capsid. Antibody responses against the capsid and transgene product will be measured using a cell-based neutralization assay and an enzyme-linked immunosorbent assay (ELISA), respectively.

Refer to the Study Operations Manual for details on the shipping and handling of samples.

Remaining samples may be stored for up to 15 years or per local regulations.

11.5 Vital Signs

Vital signs (pulse and systolic and diastolic blood pressure) will be taken at the times indicated in Table 1. Vital signs should be taken after the subject is seated for at least 5 minutes.

11.6 Best-Corrected Visual Acuity

To evaluate changes in BCVA over the study period, BCVA will be assessed using the ETDRS VA chart and performed for both eyes at the times indicated in Table 1.

The BCVA test should be performed prior to pupil dilation, and distance refraction should be carried out before BCVA is measured. Initially, letters are read at a distance of 4 metres from the chart. If <20 letters are read at 4 metres, testing at 1 metre should be performed. BCVA is to be reported as number of letters read correctly by the subject.

For BCVA, assessors will be appropriately qualified for conducting the assessment.

If a subject is not able to perform an assessment due to poor VA, it will be documented accordingly in the eCRF and will not be recorded as a protocol deviation.

11.7 Microperimetry

Microperimetry will be conducted on both eyes at the times indicated in Table 1.

Microperimetry will be conducted by certified technicians to assess changes in retinal sensitivity within the macula. All microperimetry images will be sent by the sites to a Central Reading Centre (CRC) for review. For complete technical specifications for microperimetry, refer to the Study Operations Manual (which will include procedures from the CRC regarding how measurements are to be taken).

11.8 Fundus Autofluorescence

To assess changes in the area of viable retinal tissue, fundus AF will be performed on both eyes at the times indicated in Table 1.

All fundus AF images will be performed by certified technicians at the site after dilation of the subject's pupil and sent to a CRC for review; the CRC will enter the data into the electronic data capture (EDC) system. For complete technical specifications for AF, refer to the Study Operations Manual (which will include procedures from the CRC regarding how measurements are to be taken).

11.9 Spectral Domain Optical Coherence Tomography

SD-OCT will be performed on both eyes at the times indicated in Table 1.

SD-OCT measurements will be taken by certified technicians at the site after dilation of the subject's pupil. All OCT scans will be submitted by the sites to a CRC where the scans will be evaluated; the CRC will enter the data into the EDC system. SD-OCT will be used to quantify integrity of the ellipsoid zone and reduction in the signal from the outer nuclear layer and choroid. In addition, foveal changes will be assessed. For complete technical specifications for SD-OCT, refer to the Study Operations Manual (which will include procedures from the CRC regarding how measurements are to be taken).

11.10 Full Ophthalmic Examination

A full ophthalmic examination will be performed for both eyes at the times indicated in Table 1.

Each ophthalmic examination will include IOP, slit lamp examination, lens opacity grading, and dilated ophthalmoscopy. The same slit lamp machine and lighting conditions should be used across study visits for any given subject.

In addition to the parameters listed above, subjects will be carefully examined for the presence of intraocular inflammation after vector administration. Cataract can also develop as a result of the vitrectomy procedure and can potentially affect VA. Pre-operative grading of lens opacity and colour should therefore be documented by the established clinical LOCS III. A recent study has shown that cataract surgery is effective in subjects with CHM and without any specific risks (Edwards, Jolly et al. 2016). Subjects who develop cataracts may undergo cataract surgery if deemed clinically necessary. If cataract surgery is performed, it should be carried out at least 4 weeks before Month 12 (Visit 9) for the respective eye.

11.11 7-Field Colour Fundus Photography

Seven-field colour fundus photography will be performed for both eyes at the times indicated in Table 1.

Fundus photography will be performed by certified technicians following pupil dilation. Stereo photos should be performed for fields 1, 2 and 3. All fundus photographs will be sent by the sites to the CRC for review; the CRC will enter the data into the EDC system. For complete technical specifications for fundus photography, refer to the Study Operations Manual.

12 ASSESSMENT OF EFFICACY

The following assessments are primary safety assessments that will also be considered secondary efficacy endpoints.

12.1 Best-Corrected Visual Acuity

See Section 11.6.

12.2 Fundus Autofluorescence

See Section 11.8.

12.3 Spectral Domain Optical Coherence Tomography

See Section 11.9.

12.4 Microperimetry

See Section 11.7.

13 STATISTICAL CONSIDERATIONS

13.1 Sample Size

Approximately 60 subjects are planned to be enrolled in the study. A total of 120 treated eyes is considered sufficient to characterise the immunological profile of bilateral AAV2-REP1 administration, which is the key concern of gene therapy re-administration.

13.2 Procedure for Accounting for Missing Data

All reasonable efforts will be made to obtain complete data for both eyes on all subjects. However, missing observations may occur. Management of dropout and missing observations will depend on their nature and frequency and will be discussed as part of the Data Review meeting before database lock.

Safety data will be analysed on observed data (missing data will not be imputed). The analysis of efficacy endpoints will be performed using the last observation carried forward approach for imputation of missing data.

13.3 Analysis Sets

The 'All Treated Subjects' Analysis Set will consist of all subjects who attend the 'Day of Surgery' visit of Period 1 (or, of Period 2, for subjects who received AAV2-REP1 treatment in SE1 in an antecedent study). The All Treated Subjects Analysis Set will be the primary population for demographics, baseline characteristics, and safety and efficacy analyses.

13.4 Descriptive Statistics and Conventions

Detailed specifications of the planned analyses will be included in a separate Statistical Analysis Plan (SAP).

Continuous variables (including changes from Baseline) will be summarised over time using descriptive statistics (i.e., mean, standard deviation, 95% confidence interval [CI], median, first and third quartiles, fifth and ninety-fifth percentiles, minimum, and maximum). Categorical variables (including shifts from Baseline) will be described over time using counts, percentages, and 95% CIs.

Baseline is defined, for each eye and each period, as the last non-missing value prior to treatment of the respective period, in the respective eye.

13.5 Demographics and Baseline Characteristics

Demographics will be summarised for all subjects combined. Age at the date of the Period 1 informed consent will be calculated. Baseline ocular characteristics will be summarised by eye and period.

13.6 Safety Analyses

No statistical tests will be performed.

13.6.1 Visual Acuity

See Section 13.7.1.

13.6.2 Ophthalmic Examination

IOP and change from Baseline in IOP will be summarised by eye, period and visit. Abnormal slit lamp examination findings and dilated ophthalmoscopy findings will be summarised by eye, period and visit. Lens opacity categories and shifts from baseline will be summarised by eye, period and visit.

13.6.3 Spectral Domain Optical Coherence Tomography

See Section 13.7.2.

13.6.4 Fundus Autofluorescence

See Section 13.7.3.

13.6.5 7-Field Colour Fundus Photography

Categories of colour fundus photography findings will be summarised by eye, period and visit.

13.6.6 Microperimetry

See Section 13.7.4.

13.6.7 Adverse Events

AEs will be coded using the Medical Dictionary for Regulatory Affairs, version 21.0 or higher. Events will be summarised by system organ class and preferred term and eye. The number of eyes with an AE, as well as the number of events, will be summarised, by period and eye. Similar summaries will be produced for study drug/procedure-related AEs, AEs leading to discontinuation, and SAEs. AEs will also be summarised by maximum severity, relationship to study drug/procedure, and time to onset and resolution.

13.6.8 Immunogenicity and Vector shedding

Vector shedding and immune response profiles will be described.

13.6.9 Vital Signs

Vital signs will be summarised by period and visit.

13.7 Efficacy Analyses

13.7.1 Visual Acuity

The proportion of subjects with a ≥10- and ≥15-letter improvement from baseline in BCVA will be summarised by eye, period and visit. Mean BCVA and mean change from Baseline in BCVA will be tabulated by eye, period and visit. The proportion of subjects with a ≥10- and ≥15-letter improvement from baseline in BCVA will be summarised by eye, period and visit. Mean BCVA and mean change from Baseline in BCVA will be tabulated by eye, period and visit.

13.7.2 Spectral Domain Optical Coherence Tomography

Vitreo-macular Interface Disease, Intraretinal Hyper-Reflective Spots, Cystoid Macular Edema, Subretinal Fluid, Subretinal Hyper-reflective Material and Pigment Epithelial Detachment will be summarised by eye, period and visit, as well as shifts from baseline.

13.7.3 Fundus Autofluorescence

Total Area of Preserved AF and Distance from Foveal Center to nearest Border of Preserved AF, and their change from baseline, will be summarized by eye, period and visit.

13.7.4 Microperimetry

Fixation Location and Stability, and their shift from baseline, will be summarised by eye, period and visit. Bivariate Contour Ellipse Area and Mean Sensitivity, and their change from baseline, will be summarised by eye, period and visit. An analysis specific to the Sensitivity Points will be performed, the details of which will be described in the GEMINI SAP.Any additional categorical/continuous efficacy endpoints will be summarised using descriptive statistics, with 95% 2-sided CIs calculated where appropriate. Shift from baseline will be summarised by eye and by visit, where applicable.

14 INFORMED CONSENT, ETHICAL REVIEW AND REGULATORY CONSIDERATIONS

14.1 Informed Consent

Male subjects with CHM who meet all of the entry criteria will be invited to take part in the study. Subjects must personally sign and date the latest IEC / IRB approved version of the informed consent form before any study-specific procedures are performed.

Written and verbal versions of the subject information and informed consent will be presented to the subjects detailing no less than: the exact nature of the study; the implications and constraints of the protocol; and the known side effects and any risks involved in taking part. It will be clearly stated that the subject 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.

The subject will be allowed as much time as needed to consider the information and the opportunity to question the Investigator, their primary care physician/general practitioner or other independent parties to decide whether they will participate in the study. Written informed consent will then be obtained by means of subject dated signature and dated signature of the person who presented and obtained the informed consent. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Investigator. A copy of the signed informed consent will be given to each subject. The original signed form will be retained at the study site and an additional copy will remain in the subject's medical records.

14.2 Ethical/Regulatory Review

The protocol, informed consent form, subject information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (IEC or IRB), regulatory authorities and host institution(s) for written approval. If there are any changes to the approved protocol (with the exception of emergency modifications required for the subject's safety), a protocol amendment will be issued by the Sponsor. When required by local law, the IEC/IRB and Competent Authority must give written approval of any amendments likely to affect the safety of subjects or study conduct. Each site must maintain accurate and updated records of all correspondence with the IEC/IRB.

14.3 Regulatory Considerations

The study will be conducted in full conformity with all applicable laws and regulations, including the International Council on Harmonisation (ICH) Guidelines for Good Clinical Practice (GCP) (CPMP/ICH/135/95).

The study, where permissible, will be conducted in accordance with the relevant articles of the Declaration of Helsinki as adopted by the 18th World Medical Assembly in 1964 and as revised in Tokyo (1975), Venice (1983), Hong Kong (1989), South Africa (1996), Scotland (2000), Washington (2002), Tokyo (2004), Seoul (2008), and Brazil (2013)

15 ADMINISTRATIVE PROCEDURES

15.1 Data Quality Control and Assurance

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.

The Sponsor and its selected vendors have systems in place for implementing and maintaining quality assurance and quality control systems, with written standard operating procedures (SOPs) to ensure that all aspects of the trial will be conducted in compliance with this protocol and data will be generated, documented and reported in compliance with this protocol.

Data will be entered into a validated clinical study database and subject to programmed validation checks and manually verified for accuracy and completeness by a Sponsor's representative, both remotely and during on-site monitoring visits. Any discrepancies will be resolved with the Investigator or designee, as appropriate.

Regular monitoring will be performed by the Sponsor or its designee according to ICH GCP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written SOPs, the monitors will verify that the study is conducted and data are generated, documented and reported in compliance with the protocol, ICH GCP and applicable regulatory requirements.

In addition, this study will be subject to quality assurance audits in order to independently verify compliance with the protocol.

15.2 Data Handling and Records Management

The Investigator must maintain adequate and accurate source documents, which will be the basis of information for the eCRFs. The source documents are to be separate and distinct from the eCRFs. All study data will be entered on an encrypted EDC system with pass-codes known to all Investigators and appropriately delegated study team members. This electronic data entry system has been validated. Incomplete or inconsistent data will result in data queries that require resolution by the Investigator or designee.

The Investigator must ensure that clinical study records are retained according to national regulations. The Investigator must immediately inform the Sponsor if any documents are to be destroyed, transferred to another facility or transferred to a different owner.

In addition, files containing photos or digital outputs will be electronically transmitted to the reading centre for centralised, standardised review. One reading centre is anticipated. Data from the reading centre will either be entered into the EDC system or provided as an external data set that will be loaded directly into the study database.

Samples will also be provided to a central laboratory for analysis of vector shedding and immunogenicity. Results from these analyses will also be provided as an external data set that will be loaded directly into the study database.

The Investigator must retain sufficient documentation that these images, outputs and samples were handled and transmitted appropriately.

15.3 Access to Source Documentation and Subject Privacy

Direct access will be granted to authorised representatives from the Sponsor (or designee), host institution, the IEC/IRB, and regulatory authorities to permit trial-related monitoring, audits and inspections.

The trial staff will ensure that the subject's anonymity is maintained. All documents will be stored securely and only accessible by trial staff and authorised personnel. Subjects will be identified by a subject ID number on the eCRF and any electronic database. The subject's name and any other identifying detail will NOT be included in any study data electronic file. The study will comply with the data protection laws which require data to be anonymised.

Subject medical information obtained in this study is confidential, and disclosure to third parties other than those noted below is prohibited. As required by Personal Information Protection and Electronics Documents Act and Personal Health Information Protection Act, upon the subject's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. Data generated by this study must be available for inspection by regulatory agencies, national and local health authorities, the Sponsor or their representative, and the IEC/IRB.

15.4 Time and Schedule of the Study

The estimated overall study duration is 12 months of follow-up per eye.

15.5 Policy for Publication and Presentation of Data

The detailed procedures for publications and data presentations are set out in the clinical trial agreement entered into with the Sponsor (or designee) in connection with this study.

16 APPENDIX

Table 1 presents a schedule of study procedures.

Table 1: Schedule of Study Procedures

Visit	Period 1 Screening[a]	Period 2 Baseline[b] (<10 Weeks from Visit 1, Period 2)	Study Period 1 / Period 2									ET Visit[d]	Unscheduled Visit[e]
			V1	V2	V3	V4	V5	V6	V7	V8	V9		
Study Day/Month Visit Window			**Day 0 Injection Day[c] (≤10 weeks Screening)**	**Day 1 Post op**	**Day 3 +2 d**	**Day 7 - 1/+2 d**	**Day 14 ± 3d**	**M 1 ± 7d**	**M 3 ± 14d**	**M6 ± 14d**	**M 12 ± 14d**		
Assessment/Procedures (All subjects/eyes)													
Informed Consent	X												
Demography, medical and ocular history	X												
Vital signs (pulse, blood pressure)	X	X		X	X						X	X	
Weight[f]	X	X											
Vector shedding sampling[g]	X	X		X	X	X	X	X	X			X[h]	
Immunogenicity sampling[i]	X	X		X		X	X	X	X	X	X	X	
BCVA	X	X		X	X	X	X	X	X	X	X	X	X
Microperimetry	X	X						X	X	X	X	X	
Full ophthalmic exam[j]	X	X	X	X	X	X	X	X	X	X	X	X	X
SD-OCT	X	X		X		X	X	X	X	X	X	X	X
Autofluorescence	X	X				X	X	X	X	X	X	X	
7-field colour fundus photos[k]	X	X									X	X	
Study drug / sub-retinal injection / vitrectomy / retinal detachment			X										
AE/SAE monitoring[l]	X	X	X	X	X	X	X	X	X	X	X	X	X

Visit	Period 1 Screening[a]	Period 2 Baseline[b] (<10 Weeks from Visit 1, Period 2)	Study Period 1 / Period 2									ET Visit[d]	Unscheduled Visit[e]
			V1	V2	V3	V4	V5	V6	V7	V8	V9		
Study Day/Month Visit Window			Day 0 Injection Day[c] (≤10 weeks Screening)	Day 1 Post op	Day 3 +2 d	Day 7 - 1/+2 d	Day 14 ± 3d	M 1 ± 7d	M 3 ± 14d	M6 ± 14d	M 12 ± 14d		
Assessment/Procedures (All subjects/eyes)													
Concomitant medication, procedures, and treatment review[m]	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: AE=adverse event; BCVA=best corrected visual acuity; ET=early termination; IOP=intraocular pressure; LOCS=lens opacities classification system; SAE=serious adverse event; SD-OCT=spectral domain optical coherence tomography; SE1=Study Eye 1; SE2=Study Eye 2

[a] Screening Visit/Period must be performed ≤10 weeks of Period 1, Visit 1. Selection of SE1 and SE2 will be determined by the Investigator in collaboration with the subject.

[b] Baseline assessments must occur before the SE2 is dosed. These can occur at a regularly scheduled visit if that visit is within 10 weeks prior to surgery in SE2. If it has been more than 10 weeks since a visit, the subject must return for assessments at this Baseline Period 2 visit.

[c] Subjects will undergo vitrectomy and AAV2-REP1 administration to SE1 in Period 1; SE2 in Period 2.

[d] ET visit is to be performed if a subject discontinues at any time.

[e] If clinically indicated, subjects may need to return to the site for an unscheduled visit. At a minimum, the following assessments will be performed: BCVA, full ophthalmic examination, SD-OCT, AF, concomitant medication, procedures, and treatment review and AE/SAE monitoring.

[f] Weight is collected for dose calculation of the corticosteroid regimen.

[g] Blood, tears (both eyes), urine and saliva samples.

[h] If ET visit occurs within 3 months post-treatment.

[i] Immunoassays are planned to assess cell-based and antibody-based immune responses against AAV2-REP1. Enzyme-linked immunospot (ELISPOT) assays will be used for the assessment of T-cell-mediated immune responses to transgene product (REP1) and the capsid. Antibody responses against the capsid and transgene product will be measured using a cell-based neutralization assay and an enzyme-linked immunosorbent assay (ELISA), respectively. All immunogenicity samples will be sent to a central laboratory for analysis.

[j] The ophthalmic examination will include IOP, slit lamp examination, lens opacity grading, and dilated ophthalmoscopy. The same slit lamp machine and lighting conditions should be used across the study for each subject.

[k] Stereo photos for fields 1, 2, 3.

[l] AEs/SAEs will be collected from the time the subject provides written informed consent (Screening Visit) through Period 2, Visit 9 (or ET Visit, if applicable).

[m] Subjects will be given a course of oral corticosteroid before each surgical visit, and instructed to start taking the drug 2 days prior to SE1/SE2 treatment, unless the SE2 surgical treatment coincides with the SE1 steroid treatment period, when an extended course of steroid can cover both surgical treatments. This regimen is outlined in detail in Section 9.8.

For SE1, the Full Corticosteroid Regimen should be initiated as described. For SE2, and surgeries that occur on day 21 or later, the Full Corticosteroid Regimen should be initiated as described, beginning 2 days before the scheduled SE2 surgery. If SE2 is to be performed prior to Study Day 21, then the Full Corticosteroid Regimen should be initiated 2 days prior to the scheduled surgery for SE2, and this will supersede the steroid taper in progress for SE1. The full 21 days of treatment will then be completed for SE2.

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AMENDMENT SUMMARY

Biogen Protocol 273CH203 (Formerly NSR-REP-02)

An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)

GEMINI Study

For Global Protocol Amendment: Version 6.0

Date: 12 November 2020

EUDRA CT Number: 2017-002395-75

Version 6.0 of the protocol has been prepared for this amendment, which supersedes Version 5.0 dated 30 April 2020.

PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 273CH203 (NSR-REP-02) is for safety of participants: a maximum dose of 80 mg daily of corticosteroid has been added to the corticosteroid regimen.

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

The synopsis was revised to reflect changes made throughout the protocol.

Section 7.2 Discussion of Design

Now reads:

The corticosteroid regimen is 1 mg/kg/day **(for a maximum of 80 mg/daily)** prednisone/prednisolone for a total of 10 days (beginning 2 days before the vector injection, on the day of injection, and then for 7 days); followed by 0.5 mg/kg/day for 7 days; 0.25 mg/kg/day for 2 days; and 0.125 mg/kg/day for 2 days (21 days in total).

Rationale: A maximum dose of 80 mg daily of corticosteroid has been added to the corticosteroid regimen.

This change also affects:

Section 9.8, Concomitant Therapy

The corticosteroid regimen is 1 mg/kg/day prednisone/prednisolone **(not to exceed 80 mg/daily)** for a total of 10 days (beginning 2 days before the vector injection, on the day of injection, and then for 7 days); followed by 0.5 mg/kg/day for 7 days; 0.25 mg/kg/day for 2 days; and 0.125 mg/kg/day for 2 days (21 days in total; Study Day (SD) -2 through SD18).

Full Corticosteroid Regimen

Prednisone or prednisolone administered as follows:

- SD -2 through SD 7 (10 days): 1 mg/kg by mouth daily **(not to exceed 80 mg/daily)**
- SD 8 through SD 14 (7 days): 0.5 mg/kg/day by mouth daily
- SD 15 through SD 16 (2 days): 0.25 mg/kg/day by mouth daily
- SD 17 through SD 18 (2 days): 0.125 mg/kg/day by mouth daily

Rationale: For safety reasons a maximum of 80 mg daily of corticosteroid has been added to the corticosteroid regimen.

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Not Applicable

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- Biogen protocol number was added.
- Sponsor information was updated.
- Sponsor approval page personnel was updated.
- Patient Eligibility Review Committee was added to the Synopsis Exclusion Criteria

LIST OF ABBREVIATIONS

Not Applicable

SUMMARY OF CHANGES FOR CLINICAL STUDY PROTOCOL NSR-REP-02

AAV2-REP1

An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)

GEMINI Study

Indication:	Choroideremia
Study Phase:	2
Sponsor:	NightstaRx Ltd 2nd Floor 10 Midford Place London W1T 5BJ, UK Telephone: +44 (0) 020 7062 2777
Summary of Changes:	Protocol Amendment 4.0, Version 5.0 30 Apr 2020

CONFIDENTIALITY STATEMENT

SUMMARY OF CHANGES SPONSOR APPROVAL PAGE

Clinical Study Protocol Number: NSR-REP-02

Protocol Title: An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)

Summary of Changes for Protocol: Version 5.0 30 Apr 2020

Approved By:

The person listed below is authorised to sign the summary of changes on behalf of NightstaRx Ltd/Biogen.

[redacted]
Digitally signed by [redacted]
DN: cn=[redacted], o=Biogen, ou=Ophthalmology DU, email=[redacted] c=US
Date: 2020.04.30 21:10:37 -04'00'

_____ Date: _____

[redacted], MD, PhD

[redacted]

Overview / Rationale:

The change to this protocol triggering Version 5.0 was to reference the Patient Eligibility Review Process. The protocol now clearly describes that the Investigator as well as the Sponsor and a surgical consultant through a collective decision-making process outlined in the Patient Eligibility Review Plan (version 2.0, dated 03 April 2020) ultimately determine the subject's eligibility for surgery. This process takes places subsequent to the subject's eligibility determination based on meeting inclusion and exclusion criteria and undergoing baseline assessments. The aim of the Patient Eligibility Review is to determine if the subject is an appropriate candidate for surgery through examination of baseline imaging (optical coherence tomography, fundus autofluorescence, fundus photography), best-corrected visual acuity, and microperimetry data, as well as genetic test results, taking into consideration disease progression and the status of the choroideremic retina. Both the Data Monitoring Committee for the GEMINI study, and the principal investigators have been consulted regarding this amendment, and both parties did not recommend further amending the protocol to include specific surgical eligibility criteria, preferring the current collaborative Patient Eligibility Review process.

The Patient Eligibility Review Plan and each subject's signed GEMINI surgery authorization form lie within the Trial Master File for the GEMINI study.

Summary of Changes Protocol NSR-REP-02 Version 5.0, 30 Apr 2020			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Header	~~Version 4.0 30 Oct 2019~~	Version 5.0 30 Apr 2020	Updated text to latest protocol
Title Page		Amendment 4.0, Version 5.0 30 Apr 2020	Added latest protocol
Sponsor Approval, Page 2	~~19 February~~ [redacted]	30 Apr 2020 [redacted], MD, PhD	Updated approval page date and personnel to version 5.0
	The person listed above is authorized to ~~sign~~ the protocol on behalf of NightstaRx Ltd. The~~ wet ink~~ signature is on file and available upon request.	The person listed above is authorized to approve the protocol on behalf of NightstaRx Ltd/Biogen. A signature is on file and available upon request.	The current approval process is electronic.
Investigator Signature Page, Page 3	~~19 February~~	30 Apr 2020	Updated signature page date to version 5.0
Synopsis Study Design, Pages 5 & 6		Study eye selection and eligibility for surgery will then be confirmed collectively by the Patient Eligibility Review Committee, which includes the Investigator, a consulting surgeon, and the Sponsor (see the Patient Eligibility Review Plan).	Added text to include study eye selection and eligibility for study is defined by the Patient Eligibility Review Committee
	~~Committee investigator and after consultation with the sponsor~~	Eligibility for SE2 surgery will then be confirmed collectively by the Patient Eligibility Review Committee, which includes the Investigator, a consulting surgeon, and the Sponsor (see the Patient Eligibility Review Plan).	
	If, at that time, the BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the ~~Investigator and after consultation with the sponsor~~:	If, at that time, the BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the Patient Eligibility Review Committee:	
Synopsis Exclusion Criteria Inclusion #4 Page 7		• who, in the clinical opinion of the Patient Eligibility Review Committee or Investigator, is not an appropriate candidate for sub-retinal surgery	Added text to reference the Patient Eligibility Review Committee
Section 7.1 Overall Study Design Page 17		Study eye selection and eligibility for surgery will then be confirmed collectively by the Patient Eligibility Review Committee, which includes the Investigator, a consulting surgeon, and the	Added text to include study eye selection and eligibility for study is defined by the Patient

Summary of Changes Protocol NSR-REP-02 Version 5.0, 30 Apr 2020			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		Sponsor (see the Patient Eligibility Review Plan).	Eligibility Review Committee
Section 7.1 Overall Study Design Page 18		Study eye selection and eligibility for surgery will then be confirmed collectively by the Patient Eligibility Review Committee, which includes the Investigator, a consulting surgeon, and the Sponsor (see the Patient Eligibility Review Plan).	Added text to include study eye selection and eligibility for study is defined by the Patient Eligibility Review Committee
	If, at that time, the BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the ~~Investigator and after consultation with the sponsor~~	If, at that time, the BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the Patient Eligibility Review Committee	
Section 8.2 Exclusion Criteria #4 Page 20		Patient Eligibility Review Committee or Investigator	Added text to include Patient Eligibility Review Committee
		• who, in the clinical opinion of the Patient Eligibility Review Committee or Investigator, is not an appropriate candidate for sub-retinal surgery (see the Patient Eligibility Review Plan)	
Section 10.1 Screening Period Page 26 & 28		Study eye selection and eligibility for surgery will then be confirmed collectively by the Patient Eligibility Review Committee, which includes the Investigator, a consulting surgeon, and the Sponsor (see the Patient Eligibility Review Plan).	Added text to include study eye selection and eligibility for study is defined by the Patient Eligibility Review Committee
	If BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the ~~investigator and after consultation with the sponsor:~~	Eligibility for SE2 surgery will then be confirmed collectively by the Patient Eligibility Review Committee, which includes the Investigator, a consulting surgeon, and the Sponsor (see the Patient Eligibility Review Plan). If BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the Patient Eligibility Review Committee	

SUMMARY OF CHANGES FOR CLINICAL STUDY PROTOCOL NSR-REP-02

AAV2-REP1

An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)

GEMINI Study

Indication:	Choroideremia
Study Phase:	2
Sponsor:	NightstaRx Ltd 2nd Floor 10 Midford Place London W1T 5BJ, UK Telephone: +44 (0) 020 7062 2777
Summary of Changes:	Protocol Amendment 3.0, Version 4.0 30 OCT 2019

CONFIDENTIALITY STATEMENT

SUMMARY OF CHANGES SPONSOR APPROVAL PAGE

Clinical Study Protocol Number:	NSR-REP-02
Protocol Title:	An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)
Summary of Changes for Protocol:	Version 4.0 30 OCT 2019

Approved By:

The person listed below is authorised to sign the summary of changes on behalf of NightstaRx Ltd.

[redacted]_____Date:_____[redacted]

[redacted], MD

[redacted]

Overview / Rationale:

The main changes to this protocol triggering Version 4.0 were:

Visit windows changed for Visit 3 (Day 3 + 2 days, post-operative follow-up visit) and Visit 4 (Day 7 -1/+2 day); added to decrease patient burden and minimize weekend post-operative follow-ups.

New data derived from the STAR study caused a change in the Reference Safety Information and an update to the Investigator's Brochure. This information has been added to the Risk/Benefit section of the protocol.

Additional information was added to better define serious adverse events.

Summary of Changes Protocol NSR-RPGR-02 Version 4.0, 30 Oct 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Header	~~Version 3.1 19 Feb~~	Version 4.0 30 Oct 2019	Updated text to latest protocol
Title Page		Amendment 3.0, Version 4.0 30 October 2019	Added latest protocol
Sponsor Approval, Page 2	~~19 February~~	30 October	Updated approval page date to version 4.0
Investigator Signature Page, Page 3	~~19 February~~	30 October	Updated signature page date to version 4.0
Contact Information, Page 4		CLINICAL RESEARCH ORGANIZATION	Added to header
	~~Ashfield Pharmacovigilance 5003 S. Miami Blvd, Suite 500 Durham NC 27703 Email: APV.AENightstar@ashfieldpv.com +1 919 401 8003~~	Covance (Formerly known as Chiltern International, Ltd) Chiltern International Limited Covance Clinical and Periapproval Services, Ltd Osprey House, Maidenhead Office Park, Westacott Way, Littlewick Green, Maidenhead SL6 3QH, UK +44 (0) 1628 548000	Updated the pharmacovigilance information
	~~Serious Adverse Event (SAE) and Pregnancy Notification Forms should be emailed to safetyGEMINI@nightstartx.com. For further information refer to Section 11, Assessment of Safety.~~		Deleted information no longer necessary
Synopsis Study Design, Pages 5 & 6		Each study eye will be followed for at least 12 months post-treatment, for up to 9 visits per treatment period: Visit 1 (Day 0, Injection Day Visit); Visit 2 (Day 1, post-operative follow-up visit); Visit 3 (Day 3 + 2 days, post-operative follow-up visit); Visit 4 (Day 7 -1/+2 days); Visit 5 (Day 14 ± 3 days); Visit 6 (Month 1 ± 7 days); Visit 7 (Month 3 ± 14 days); Visit 8 (Month 6 ± 14 days) and Visit 9 (Month 12 ± 14 days; end of study visit).	Added to visit window to decrease patient burden and minimize weekend post-operative follow-ups.
Section 5.4 Risk/Benefit Assessment Page 15	~~Loss of VA is, therefore, considered a Potential Risk of AAV2-REP1 treatment, in addition to the above described potential and anticipated surgical risks.~~ See the Investigator's Brochure for ~~AAV2-REP1 for~~ further details.	Complications from the surgical procedure are expected and are not considered reportable as suspected unexpected serious adverse reactions (SUSARs) unless also assessed to be at least possibly related to timrepigene emparvovec.	New data derived from the STAR study caused a change in the Reference Safety Information This new information

Summary of Changes Protocol NSR-RPGR-02 Version 4.0, 30 Oct 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		The Reference Safety Information described in the current version of the Investigator's Brochure for AAV2-REP1 defines visual acuity reduced as an expected serious adverse drug reaction, with a frequency of 4/79 (5.1%), defined as common (≥1/100 to <1/10). Loss of VA is considered an identified risk of AAV2-REP1. Inflammation is a potential risk of AAV2-REP1.	has been added to the Benefit-Risk Section.
Section 7.1 Overall Study Design, Page 19		Each study eye will be followed for at least 12 months post-treatment, for up to 9 visits per treatment period: Visit 1 (Day 0, Injection Day Visit); Visit 2 (Day 1, post-operative follow-up visit); Visit 3 (Day 3 + 2 Days); Visit 4 (Day 7 -1/+2 Days); Visit 5 (Day 14 ± 3 Days); Visit 6 (Month 1 ± 7 Days); Visit 7 (Month 3 ± 14 Days); Visit 8 (Month 6 ±14 Days) and Visit 9 (Month 12 ± 14 Days; end of study visit).	Added to decrease patient burden and minimize weekend post-operative follow-ups.
Section 9.4 Vitrectomy Procedure and Injection of AAV2-REP1, Page 24		In the second step of the procedure, the BSS cannula is removed from the eye and AAV2-REP1 is prepared for injection. A dose of up to 1.0×10^11 gp of AAV2-REP1 is injected into the sub-retinal space through the same entry site.	Clarified text
Section 10.4 Period 1 and 2, Visit 3, (Post-operative Day 3 + 2), Page 29		Subjects will return to the site 1, 3, 7 and 14 days after surgery for post-operative follow-up (Visit 2 [Day 1], Visit 3 [Day 3 + 2 Days], Visit 4 [Day 7 -1/+2 Days] and Visit 5 [Day 14 ± 3 Days], respectively).	Added to decrease patient burden and minimize weekend post-operative follow-ups.
Sections 10.5 Period 1 and 2, Visit 4 (Post-operative Day 7 -1/+2 days), Page 29	10.5 Period 1 and 2, Visit 4 (Post-operative Day 7 ~~± 1~~-Days) For Visit 4 (Post-operative Day 7 ~~± 1~~-day), ocular assessments and procedures will be performed on each eye. At Visit 4 (Day 7 ± 1 -1/+2 days),	10.5 Period 1 and 2, Visit 4 (Post-operative Day 7 -1/+2 Days) For Visit 4 (Post-operative Day 7 -1/+2 days), ocular assessments and procedures will be performed on each eye. At Visit 4 (Day 7 -1/+2 days),	Added to decrease patient burden and minimize weekend post-operative follow-ups.
Section 11.1.1.2	Hospitalisation~~, including elective procedures~~…	Hospitalisation that was pre-scheduled prior to obtaining the	Added text to better define

Summary of Changes Protocol NSR-RPGR-02 Version 4.0, 30 Oct 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Serious Adverse Event, Pages 33-34		consent to participate in the study or for an elective procedure or routinely scheduled treatment for a pre-existing condition, which has not worsened, does not constitute an SAE. A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the subject is hospitalized. The study site must document all of the following: • The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the subject's consent to participate in the study. • The condition requiring the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the subject's consent to participate in the study and the time of the procedure or treatment. • The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission. If a subject is hospitalized due to local requirements for administration of the study treatment, the hospitalization should not be considered an SAE unless one of the requirements in Section 11.1.1.2 is met.	serious adverse events
Table 1 Schedule of Study Procedures	V3 Day 3 ~~Post op~~ V4 Day 7 ~~±1 d~~	V3 Day 3 +2 d V4 Day 7 -1/+2 d	Updated Table 1 headers

SUMMARY OF CHANGES FOR CLINICAL STUDY PROTOCOL NSR-REP-02

AAV2-REP1

An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)

GEMINI Study

Indication:	Choroideremia
Study Phase:	2
Sponsor:	NightstaRx Ltd 2nd Floor 10 Midford Place London W1T 5BJ, UK Telephone: +44 (0) 020 7062 2777
Summary of Changes:	Protocol Version 3.0 4 Feb 2019

CONFIDENTIALITY STATEMENT

SUMMARY OF CHANGES SPONSOR APPROVAL PAGE

Clinical Study Protocol Number:	NSR-REP-02
Protocol Title:	An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)
Summary of Changes for Protocol:	Version 3.0 4 Feb 2019

Approved By: [redacted], MD

The person listed above is authorised to sign the summary of changes on behalf of NightstaRx Ltd. A wet ink signature is on file and available upon request.

Overview / Rationale of Major Changes:

This protocol amendment was triggered primarily by recommendations and interactions from regulatory bodies that have occurred in 2018. Five key revisions were made: 1) the sample size was increased from 15 to 60; 2) specific language was added regarding the allocation of these 60 subjects into 3 surgery windows of varying length, as recommended by FDA, to provide the broadest safety profile available on subjects treated with short, medium and long time periods between surgeries; 3) the corticosteroid regimen was revised to clarify administration required with bilateral treatment performed with very short windows between surgeries; 4) the visual acuity inclusion criterion was revised to allow for the eligibility of subjects with more significant visual impairment; and lastly, 5) new requirements have been added to mandate baseline assessments prior to treating the second eye in the case of long surgery windows, to assure that the second eye remains eligible and that the investigator still recommends treatment based on safety assessments and the visual function of both eyes at that time, as well as to establish accurate baseline values. In addition, a new inclusion criterion has been added mandating that subjects who enter this study from an antecedent study must have biological samples available to complete an adequate immunogenicity profile, if needed.

A new benefit-risk assessment section has also been added to the Introduction, based on information collected from ongoing safety reporting of the Phase 3 STAR study. This new section outlines the expected risks of AAV2-REP1 treatment to include visual acuity loss post-treatment. The definition of vision loss as a serious adverse event was clarified, without changing the original meaning. Adverse event reporting was also modified to a binary system that still allowed the investigator to choose 3 levels of relatedness under “related to study drug or procedure”.

Minor changes were made solely for clarification.

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Title Page	~~Version 2.0 17 May 2018~~	Version 3.0 1 Feb 2019	Updated document header
		CT Number: NCT03507686 INN: Timrepigene emparvovec Amendment 2 1 February 2019	Added to title page
	~~Wellcome Gibbs Building, 215 Euston Road London NW1 2BE, UK 207 611 2077~~	2nd Floor 10 Midford Place London W1T 5BJ UK 020 7062 2777	Change of address and telephone
Sponsor Approval, Page 2	~~17 May 2018~~	1 February 2019	Updated to date of protocol version 3.0
	~~[redacted], MD~~	[redacted], MD	Personnel change
	~~The person listed below is authorised to sign the protocol on behalf of NightstaRx Ltd. Signed: Date: [redacted], MD~~	The person listed above is authorised to sign the protocol on behalf of NightstaRx Ltd. The wet ink signature is on file and available upon request	Removed signature line from sponsor approval page and added referral for wet signature
Investigator Signature Page, Page 3	~~17 May 2018~~	1 February 2019	Updated protocol date to version 3.0
		(where required)	Clarified text
Contact Information, Page 4	[redacted] ~~NightstaRx Ltdr Wellcome Gibbs Building, Euston Road London NW1 2BE, UK~~ [redacted] ~~NightstaRx Ltd Wellcome Gibbs Building, Euston Road London NW1 2BE, UK~~ [redacted] [redacted] ~~Medical Monitor~~ [redacted], ~~MD~~ ~~NightstaRx Ltd Wellcome Gibbs Building, 215 Euston Road London NW1 2BE, UK~~		The contact page within the protocol now includes only pharmacovigilance contact and the responsible CRO. The complete study contact list will be included in the study manual.

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~Pharmacovigilance [redacted], MD NightstaRx Ltd Wellcome Gibbs Building, 215 Euston Road London NW1 2BE, UK~~	Pharmacovigilance Ashfield Pharmacovigilance 5003 S. Miami Blvd, Suite 500 Durham NC 27703 Email: APV.AENightstar@ashfieldpv.com +1-919-401-8003	Contact information changed
	~~Contract Research Organization Chiltern International, Ltd 171 Bath Road, Slough, Berkshire, SL1 4AA, UK +44 (0) 175 351 2000~~	Contract Research Organization (CRO) Covance Clinical and Periapproval Services, Ltd Osprey House Maidenhead Office Park, Westacott Way, Littlewick Green Maidenhead SL6 3QH, UK	
		Additional contact information is available in the Site Operations Manual. For further information refer to Section 11, Assessment of Safety.	Added text for contact information
Synopsis Study Period (years), Page 5		Approximately 24 months (12 months of follow-up for each eye)	Clarified text
Synopsis Study Objective, Page 5		in adult male subjects….	Added text to clarify subject criteria in objective
Synopsis Primary Endpoints, Page 5	~~The primary endpoint is the evaluation of safety following of bilateral administration of AAV2-REP1.~~	The safety of bilateral administration of AAV2-REP1 will be evaluated with the following safety measures: •Best corrected visual acuity (BCVA) as measured by the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart •Ophthalmic examination assessments (including intraocular pressure [IOP], slit lamp examination, lens opacity grading and dilated ophthalmoscopy) •Spectral domain optical coherence tomography (SD-OCT) •Fundus autofluorescence (AF) •Fundus photography •Microperimetry •Adverse event (AE) reporting •Vector shedding post-treatment •Immunogenicity sampling post-treatment	The original protocol did not specify the exact measures to use in evaluating the safety of bilateral AAV2-REP1. Here we list al measures that will comprise the safety evaluation

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		•Vital signs.	
Synopsis Secondary Endpoints, Page 5	•Change from Baseline in ~~best corrected visual acuity~~ (BCVA) as measured by the ~~Early Treatment of Diabetic Retinopathy Study~~ (ETDRS) chart •Change from Baseline in ~~autofluorescence~~ (AF) •Change from Baseline in ~~spectral domain optical coherence tomography~~ (SD-OCT)	Secondary Efficacy Endpoints include:	Removed definitions of abbreviations previously defined in text
Synopsis Study Design, Pages 5 & 6	The study will consist of a Screening ~~Visit~~ followed by 2 treatment periods (Period 1 and Period 2) with up to 9 visits per period. ~~At~~ the Screening ~~Visit,~~ subjects will be assessed for eligibility of both eyes.	This is a multi-centre, open-label, prospective, 2 period, …, The study will consist of a Screening Period followed by 2 treatment periods (Period 1 and Period 2) with up to 9 visits per period. During the Screening Period, subjects will be assessed for eligibility of both eyes.	Added text, Clarified text
		The investigator will assign the order in which the eyes are treated (i.e., study eye 1 [SE1]-Period 1] and study eye 2 [SE2]- Period 2], respectively with a number assigned to each eye. This will be done in collaboration with the subject; however, the worse eye will generally be selected for first treatment. The estimated target interval between the surgical procedures of SE1 and SE2 will be determined at the Screening Visit.	Added text to specify period 1 and period 2, and target interval
		…Visit 4 (Day 7 ± 1 day); Visit 5 (Day 14 ± 3 days); Visit 6 (Month 1 ± 7 days); Visit 7 (Month 3 ± 14 days); Visit 8 (Month 6 ± 14 days) and Visit 9 (Month 12 ± 14 days; end of study visit).	Added text to clarify visit windows
		…, in which case, the study visits will follow the schedule for SE2.	Clarified procedure
	The interval between SE1 and SE2 treatment is expected to vary ~~subject from a few weeks to many months.~~	The interval between SE1 and SE2 treatment is expected to vary among subjects from weeks to many months.	Clarified text

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~Each site will work with the Sponsor and the assigned Contract Research Organisation (CRO) to try and ensure an even spread of subjects across the intervals.~~	Thus, approximately 20 subjects each will be treated with a short (<6 month), medium (6-12 months), or long (>12 month) surgery window between treatment of the first and second eye.	Specified allocation of subjects into 3 surgery windows as recommended by FDA to mimic real-world application of drug and to provide a broad assessment of safety with bilateral treatment.
	Because the timing of the second surgery visit (Period 2, Visit 1) will vary ~~between~~ subjects, the duration of study Period 1 will also be variable. ~~To avoid unnecessary study visits, every effort should be made by the investigator to schedule Period 2, Visit 1 to coincide with a planned Period 1 post-treatment visit.~~	Because the timing of the second surgery visit (Period 2, Visit 1) will vary among subjects the duration of study Period 1 will also be variable.	Clarified text, removed excess text in synopsis
		or other post-operative sequelae,	Expanded text
		…the Investigator will determine the eligibility of the subject and decide the appropriate time to schedule SE2, Visit 1.	
		All baseline ophthalmic assessments must be conducted within 3 months of SE2 surgery (Period 2, Visit 1). If, at that time, the BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the investigator and after consultation with the sponsor: 1) the subject is found eligible by all other inclusion and exclusion criteria, and 2) the subject is considered an appropriate candidate who has a potentially modifiable disease .	Mandated that baseline assessments be conducted if the second eye is done with a long time period between surgeries to assure that the eligibility and safety of treating the second eye and the overall safety and visual function of the subject.
		Post treatment subjects will no longer follow the Period 1 visit schedule….	Clarified text, added text to clarify procedure for cataract surgery

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		The cataract surgery timing must be discussed with the medical monitor prior to the procedure.	
Synopsis Number of Subjects, Page 6	~~At least 15~~ subjects are planned for the study.	Approximately 60 subjects are planned for the study.	Updated number of subjects planned
Synopsis Inclusion Criteria, Pages 6 & 7	~~At~~ the Screening ~~Visit,~~ subjects ~~are~~ found eligible for study participation if they meet all of the following inclusion criteria	During the Screening Period, subjects will be found eligible for study participation if they meet all of the following inclusion criteria	Clarified text
	≥34 ETDRS letters (~~equivalent to better than or equal 20/40 or better Snellen acuity) and ≤≥74 73 ETDRS letters (equivalent to better than or equal to 6/9 or 20/32 Snellen, decimal 0.63, LogMar 0.2~~) in both eyes1 ~~or both untreated eyes*, if no prior treatment with AAV2-REP1 o≥74 ETDRS letters (equivalent to better than or equal to 6/9 or 20/32 Snellen, decimal 0.63, LogMar 0.2) in the untreated eye, if prior treatment with AAV2-REP1 was received in an alternative study*~~	3.Have documentation of a genetically-confirmed diagnosis of CHM 5. Have a BCVA of ≥34 ETDRS letters (20/200 or better Snellen acuity) in both eyes, or in the untreated eye, if the other eye was previously treated with AAV2-REP1* * If previously treated with AAV2-REP1 in an antecedent study, subjects may be eligible for participation following Sponsor approval. 6. For subjects who received treatment with AAV2-REP1 in an antecedent study, have biological samples available to complete an adequate immunology profile.	Updated inclusion criteria to specify criteria for CHM diagnosis and eye for BCVA, added criteria for subjects in a previous study. See Rationale.
Synopsis Exclusion Criteria, Page 7	At the Screening Visit, subjects ~~are~~ found not eligible…	At the Screening Visit, subjects will be found not eligible…, 2. …or abstain from sexual intercourse… 5. …in an antecedent study with AV2-REP1.	Added text to further explain exclusion criteria
Synopsis Test Product, Dosage, and Mode of Administration, Page 7	…~~receive~~ 0.1 mL ~~sub-retinal injection~~ of study drug containing 1 × 10^11 AAV2-REP1 genome particles.	…receive a sub-retinol injection of up to 0.1 mL of study drug containing 1 × 10^11 AAV2-REP1 genome particles (gp)	Clarified text
Synopsis Criteria for Evaluation, Page 7	The safety evaluation will be based full ophthalmic examination (including ~~intraocular pressure [~~IOP~~]~~, slit lamp examination, lens opacity grading and dilated	The safety evaluation will be based on BCVA (as measured by the ETDRS chart); full ophthalmic examination (including IOP, slit lamp examination, lens opacity grading and dilated	Updated criteria to include BCVA, SD-OCT, AF, and microperimetry

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	ophthalmoscopy), fundus photography; adverse event (AE) reporting; vector shedding and immunogenicity ~~sampling~~; and vital signs.	ophthalmoscopy), SD-OCT; fundus AF; fundus photography; microperimetry; adverse event (AE) reporting; vector shedding and immunogenicity; and vital signs.	
Synopsis Statistical Methodology, Page 7	median, ~~Q1, Q3, P05, P95,~~ min, and max).	median, first and third quartiles, fifth and ninety-fifth percentiles, min, and max).	Descriptive statistics clarified
	~~Both the number of subjects and the number of eyes experiencing an AE, as well as the number of events, will be summarised.~~	The number of eyes with an AE, as well as the number of events, will be summarised, by period and eye.	Clarified text for number of eyes and events summarised
Abbreviations, Page 11	~~(c)DNA (complementary) deoxyribonucleic acid~~	cDNA Complementary deoxyribonucleic acid	Clarified text
		AF autofluorescence AAV2-REP1 recombinant… SAP Statistical Analysis Plan SD study day SUSAR Suspected unexpected serious adverse reaction	Added abbreviations
Section 5.2 Gene Therapy with Adeno-Associated AAV, Page 13	~~Considering same-day bilateral administration,~~ there is total antigen exposure following ~~the 6×10^9 genome particles (gp)/eye dose administered~~ is comparable to a 1.2×10^10 gp ~~unilateral dose,~~ which equates to a 1.2×10^12 gp dose in humans, ~~providing~~ a 12-fold immunogenicity safety margin compared to the ~~maximum~~ clinical dose of 1×10^11 gp.	In another non-clinical study in rats, lack of apparent immunogenicity was observed following same-day bilateral administration of 6×10^9 genome particles (gp)/eye AAV2-REP1 (Charles River Study No. 5700437). This total antigen exposure following bilateral dosing is comparable to a unilateral dose of 1.2×10^10 gp, which equates to a 1.2×10^12 gp dose in humans. This provides a 12-fold immunogenic safety margin compared to the planned clinical dose of 1×10^11 gp.	Clarified dosing text
	In this study, 11 children and adults with Leber's congenital amaurosis received ~~one~~ single sub-retinal administration of AAV2-RPE65 at 1.5×10^11	In the clinical setting, repeat administration…, In this study, 11 children and adults with Leber's congenital amaurosis received a single sub-retinal administration of AAV2-RPE65 at a dose of 1.5×10^11…, AAV2-RPE65, voretigene neparvovec-rzyl (LUXTURNA™, Spark Therapeutics, Inc) has been approved in the US- and the EU as the first adeno-associated virus	Clarified text, Added text for further information on AAV2

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		vector type 2 (AAV2)-based gene therapy indicated for the treatment of patients with confirmed bi-allelic RPE65 mutation-associated retinal dystrophy.	
Section 5.3 Study Rational, Page 13 & 14	~~In defective lymphocytes and fibroblasts isolated from patients with CHM, delivery of full-length human REP1 via AAV2-REP1 restored RabGGTase activity. In addition,~~ AAV2-REP1 expresses high levels of human REP1 protein…	AAV2-REP1 is a recombinant AAV2 particle…,	Clarified text
	~~In 2012,~~ an investigator-sponsored, first-in-human study of AAV2-REP1 was initiated in patients with CHM ~~(MacLaren 1 study).~~	In 2011, an investigator-sponsored, first-in-human study of AAV2-REP1 was initiated in adult male patients with CHM.	Specified males
	~~Based upon these~~ favorable results ~~tThree~~ other investigator-sponsored studies were then initiated in which a total of ~~32~~ CHM patients ~~have~~ received a single sub-retinal injection of 1×10^11 gp in 1 eye ~~with AAV2-REP1 (the MacDonald, Fischer and Lam studies).~~ The initial ~~(unpublished)~~ results of these trials are consistent with improved rod and cone function ~~that overcome~~ any negative effects of retinal detachment, and the gene therapy ~~appears to be generally~~ well tolerated ~~following a single treatment with AAV-REP1~~. ~~Emerging~~ data from non-clinical and clinical studies with AAV2 vectors demonstrate that ~~AAV2 vectors~~ illicit a minimal immune response, including after bilateral administration.	Following favorable results 3 other investigator-sponsored studies were then initiated in which a total of 26 adult male CHM patients received a single sub-retinal injection of 1×10^11 gp AAV2-REP1 in 1 eye. The initial results of these trials are consistent with improved rod and cone function despite any negative effects of retinal detachment, and the gene therapy was generally well tolerated. AAV2 REP1 gene therapy has been shown to positively affect visual acuity (VA), considering both maintenance and stabilization of vision in the majority of subjects, as well as improvement of vision in subsets of CHM subjects for up to 2 years and beyond. This clinical evidence supports the durability of the therapeutic effect of AAV2-REP1 for treating CHM. These findings lend support to further assessment of AAV2 gene therapy in the treatment of CHM. NightstaRx is currently sponsoring a multi-centre, global Phase 3 study of a single subretinal administration of AAV2-REP1 for the treatment of	Clarified text, updated # of adult male CHM patients, added text to show clinical evidence of efficacy and safety

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		CHM (NSR-REP-01, the STAR study, NCT03496012). As noted, emerging data from non-clinical and clinical studies with AAV2 vectors demonstrate that these vectors illicit a minimal immune response, including after bilateral administration.	
Section 5.4 Risk/Benefit Assessment Page 14		5.4 Risk / Benefit Assessment No treatment currently exists for CHM. The non-clinical studies conducted with AAV2-REP1 showed that it provides efficient and functional transgene expression in CHM mouse and human cells, as well as in mouse and human RPE and photoreceptors, without overt toxicity. Results from the 26-week single-dose combined toxicity/biodistribution study conducted in rats indicate that administration of AAV2-REP1 by single sub-retinal injection to both eyes is well tolerated at dose levels of 1×10^9 and 6×10^9 gp/eye (equal to 1×10^11 and 6×10^11 gp/eye in humans) when evaluated 4 and 26 weeks after injection. Minor reductions in some ERG parameters, ocular inflammation, and microscopic signs of retinal/corneal degeneration were observed and were considered procedure-related or not biologically significant. In humans, application of AAV2-REP1 to the surface of the retina requires retinal detachment via vitrectomy. The 2-step procedure employed in delivering AAV2-REP1 sub-retinally (see Section 9.4), allows the management of unexpected surgical complications of retinal detachment before the AAV2-REP1 is applied. Furthermore, since the volume of fluid required to detach the fovea is variable, by excluding the vector from the first step, and	Added section for Risk Benefit Assessment with new definition of vision loss as possible risk, as recommended by regulatory body

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		administering the vector after successful retinal detachment has been achieved, a precise, consistent dose in terms of genome particles can be applied into the sub-retinal space. Nevertheless, sub-retinal injection of AAV2-REP1 carries the risks associated with vitrectomy and retinal detachment, which include intra-operative and post-operative complications; infection (most notably infectious endophthalmitis); low and elevated IOP; choroidal detachment; persistent retinal detachment, retinal tears, holes and breaks; macular hole and macular oedema; vitreous haemorrhage; visual impairment; metamorphopsia; and photopsia (Park et al., 1995; Thompson et al., 1996; Banker et al., 1997; Cheng et al., 2001; Anderson et al., 2006; Stein et al., 2009; Recchia et al., 2010). Post-operative intraocular inflammation caused by vitrectomy is often associated with transient or sometimes permanent visual impairment. Another complication of vitrectomy is cataract formation, which may require an additional surgical procedure (cataract extraction) (Park et al., 1995; Cheng et al., 2001; Recchia et al., 2010). In addition, the surgical procedure is performed under general anesthesia, which includes the risks of dizziness, confusion, nausea and vomiting. Loss of visual acuity has been observed post-treatment with AAV2-REP1.; this visual acuity loss was determined by the investigators to be plausibly related to the administration of AAV2-REP1, however without definitively attributing the cause to either the study procedure or the GTMP. Loss of visual acuity is, therefore, considered a Potential Risk of AAV2-REP1 treatment, in addition to the above described potential and	

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		anticipated surgical risks. See the Investigator's Brochure for AAV2-REP1 for further details. Thus, although there are risks associated with the administration of the study treatment via vitrectomy and retinal detachment, the potential for benefit in the form of improved visual acuity that may be provided to subjects with CHM following treatment with AAV2-REP1 provides an acceptable risk-benefit profile for participation in this study.	
Section 6.0 Study Objectives and Endpoints, Page 15		The objective of the study is to evaluate the safety of bilateral, sequential sub-retinal administration of a single dose of AAV2-REP1 in adult male subjects with CHM.	Added clarification to subject criteria
	~~The primary endpoint is the evaluation of safety following bilateral administration of AAV2-REP1.~~	The safety of bilateral administration of AAV2-REP1 will be evaluated with the following safety measures: •BCVA as measured by the ETDRS chart •Ophthalmic examination assessments (including intraocular pressure [IOP], slit lamp examination, lens opacity grading and dilated ophthalmoscopy) •Spectral domain optical coherence tomography (SD-OCT) •Autofluorescence (AF) •Fundus photography •Microperimetry •Adverse event (AE) reporting •Vector shedding post-treatment •Immunogenicity sampling post-treatment •Vital signs	Added safety assessments to endpoints. These were already in protocol but were not specified as part of the endpoint analyses
	~~Secondary endpoints include:~~	The following will also be considered secondary efficacy endpoints: •Change from Baseline in BCVA as measured by the ETDRS chart •Change from Baseline in AF •Change from Baseline in SD-OCT •Change from Baseline in microperimetry.	Clarified text, used abbreviations

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 7.1 Overall Study Design, Pages 17 & 18	This is a multi-centre, open-label, prospective, ~~two~~-period… The study will consist of a Screening ~~Visit~~ followed by 2 treatment periods (Period 1 and Period 2) with up to 9 visits per treatment period.	This is a multi-centre, open-label, prospective, 2-period, bilateral interventional safety study of AAV2-REP1…, The study will consist of a Screening Period followed by 2 treatment periods (Period 1 and Period 2) with up to 9 visits per treatment period. A second baseline visit prior to surgery of SE2 (Period 2, Visit 1) may be required.	Clarified text, added bilateral to design, added second baseline
	~~At~~ the Screening ~~Visit~~, …,	During the Screening Period…, …(i.e., study eye 1 [SE1]-Period 1] and study eye 2 [SE2]-Period 2], respectively)… The estimated target interval between the surgical procedures of SE1 and SE2 will be determined at the Screening Visit.	Clarified text
		Visit 4 (Day 7 ± 1 Day); Visit 5 (Day 14 ± 3 Days); Visit 6 (Month 1 ± 7 Days); Visit 7 (Month 3 ± 14 Days); Visit 8 (Month 6 ±14 Days) and Visit 9 (Month 12 ± 14 Days; end of study visit).	Added text to clarify visit window
		The estimated target interval between the surgical procedures in of SE1 and SE2 will be determined at the Screening Visit unless Period 2 commences during the time of follow-up for SE1; in which case, the study visits will continue following the schedule for SE2.	Updated text
	The interval between SE1 and SE2 treatment is expected to vary ~~for each~~ subject from a ~~few~~ weeks ~~to~~ many months. ~~Each site will work with the Sponsor and the assigned Contract Research Organisation (CRO) to try and ensure an even spread across the intervals. Given that~~ the timing of the second surgery visit (Period 2, Visit 1) will vary ~~between~~ subjects, the duration of study Period 1 will also be variable. ~~To~~	The interval between SE1 and SE2 treatment is expected to vary among subjects from weeks to many months. Thus, approximately 20 subjects each will be treated with a short- (<6 month), medium- (6-12 months), or long- (>12 month) surgery window between treatment of the first and second eye. Because the timing of the second surgery visit (Period 2, Visit 1) will vary among subjects, the duration of study Period 1 will also be variable.	Clarified text, added text for surgery window as recommended by FDA. See Rationale.

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~avoid unnecessary study visits, every effort should be made by the investigator to schedule Period 2, Visit 1 to coincide with a planned Period 1 post treatment visit.~~		
		Signs of post-operative inflammation or other post-operative sequelae, as judged by the investigator, should be resolved in SE1 for the subject to continue to Period 2. After comprehensive evaluation of all safety assessments and visual functional tests of SE1 and SE2, the Investigator will determine the eligibility of the subject and decide the appropriate time to schedule SE2, Visit 1.	Clarified text to be more inclusive
		All baseline ophthalmic assessments must be conducted within 3 months prior to SE2 surgery (Period 2, Visit 1). If, at that time, BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the investigator and after consultation with the sponsor: 1) the subject is found eligible by all other inclusion and exclusion criteria, and 2) the subject is considered an appropriate candidate who has potentially modifiable disease.	Updated to include baseline assessment requirements if second eye is treated with a long time period between surgeries
Subjects Previously Treated with AAV2-REP1, Pages 18 & 19	~~In these subjects, the period between SE1 (from previous study) and SE2 (current study) may exceed 6 months; prolonged intervals will not preclude subjects from being considered for study participation.~~ ~~Of note,~~ subjects who received previous treatment with AAV2-REP1~~ in an investigator-sponsored trial~~, an adequate immunology profile ~~must be available from~~ the previous study ~~for the treated eye~~.	For subjects who received previous treatment with AAV2-REP1 biological samples must be available to obtain an adequate immunology profile related to treatment of the first eye in the previous study. For subjects whose first eye was dosed in an antecedent study, and who do not have the required baseline assessments within 12 weeks prior to surgery, a Baseline Visit (see Section 10.1.1 will occur be necessary within 12 weeks prior to second eye dosing at Visit 1, Period 2.	Updated criteria for previously treated subjects in an antecedent study. This new language assures a proper baseline assessment has been conducted prior to treated second eye.

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 7.2 Discussion of Design, Page 19	Bilateral sub-retinal administration of AAV2-REP1 was explored in a pre-clinical toxicology study in rats and was found to be well tolerated and ~~safe.~~	All enrolled subjects will receive treatment with AAV2-REP1 at a dose of 1×10^11 AAV2-REP1 gp/eye. Bilateral sub-retinal administration of AAV2-REP1 was explored in a pre-clinical toxicology study in rats and was found to be well tolerated and not associated with drug-related AEs.	Added text for dose
	The planned sample size (~~at least 15~~ subjects/~~30~~ eyes)	The planned sample size (approximately 60 subjects, with a total of 120 treated eyes) …	Updated sample size
	~~This open-label safety study has no formal control arm. The inclusion criterion of BCVA ≥74 ETDRS letters ensures that the study eyes are optimal surgical candidates (i.e. earlier phase of the disease with larger area of viable retina). This will improve evaluation of immunogenicity of AAV2-REP1, including correlation between immunogenicity and AEs, by limiting surgery-related AEs and confounding variables.~~		Deleted text
	The dose of vector being employed in this study is based on previous clinical trials using the AAV2 vector with a chicken β actin (CBA) promoter (Maguire 2009; MacLaren 2014), and the ~~ongoing~~ investigator~~-driven~~ clinical studies in which AAV2-REP1~~ is being~~ administered to subjects with CHM (MacLaren 2014; MacDonald, Lam and Fischer, unpublished data).	The dose of vector being employed in this study is based on previous clinical trials using the AAV2 vector with a chicken β actin (CBA) promoter (Maguire 2009; MacLaren 2014), and the Phase 1 / 2 investigator sponsored clinical studies in which AAV2-REP1 is was administered to subjects with CHM (MacLaren 2014; MacDonald, Lam and Fischer, unpublished data).	Clarified Text
	Application of AAV2-REP1 to the retina requires retinal detachment following vitrectomy.~~ As such, sub-retinal injection of AAV2-REP1 carries the risks associated with~~	The risks of vitrectomy and retinal detachment are summarised in Section 5.4.	Moved the rsisks to the new risk benefit assessment section

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~vitrectomy and retinal detachment, which include intra-operative and post-operative complications; infection (most notably infectious endophthalmitis); low and elevated IOP; choroidal detachment; macular oedema; vitreous haemorrhage; visual impairment; metamorphopsia; and photopsia (Park 1995; Thompson 1996; Banker 1997; Cheng 2001; Anderson 2006; Stein 2009; Recchia 2010). Post-operative intraocular inflammation caused by vitrectomy is often associated with transient visual impairment. A long-term complication of vitrectomy is cataract formation, which may require an additional surgical procedure (cataract extraction) (Park 1995; Cheng 2001; Recchia 2010).~~		
	In line with guidance from regulatory agencies, the treatment intervals ~~ranging from a few weeks to ≥ 6 months apart~~ were chosen to comprehensively characterise the immunological profile following bilateral, sequential AAV2-REP1 treatment. As noted ~~in the first paragraph above~~, both preclinical data with AAV2-REP1, …	In line with guidance from regulatory agencies, the treatment intervals were chosen to comprehensively characterise the immunological profile following bilateral, sequential AAV2-REP1 treatment, and to reflect real-world application of gene therapy. As noted both preclinical data with AAV2-REP1, and clinical data with sub-retinal administration of an AAV2 vector in subjects with Leber's congenital amaurosis, support bilateral administration of AAV2-REP1 in humans.	Removed text not used in study design discussion
	~~To minimise inflammation resulting from potential immune responses to vector, including treatment of the second eye (SE2),~~	To minimise inflammation resulting from surgery and potential or unexpected immune responses to vector/transgene, all subjects will be given a course of oral	Added text to ~~update~~ clarify procedure/ regimen for all subjects to minimize

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~subjects will be given ainitiate a 21-day course of oral corticosteroid before each surgical visit, and will be instructed to start taking the drug medication 2 days prior to SE1/SE2 treatment. If the SE2 steroid treatment coincides overlaps with the SE1 steroid treatment period, an extended course of steroid treatment will be implemented to will cover both surgical treatments .~~	prednisone/prednisolone, initiated 2 days prior to surgery on both SE1 and SE2. The regimen has been modified from the 17-day protocol established in the Philadelphia AAV gene therapy clinical trial (Maguire 2008), with the allowance of an extra 4 days for tapering the dose at the end of the course. The corticosteroid regimen is 1 mg/kg/day prednisone/prednisolone for a total of 10 days (beginning 2 days before the vector injection, on the day of injection, and then for 7 days); followed by 0.5 mg/kg/day for 7 days; 0.25 mg/kg/day for 2 days; and 0.125 mg/kg/day for 2 days (21 days in total). See Section 9.8 for details.	inflammation from surgery. This was necessary due to the inclusion of short time frames between surgeries in which case steroid treatments will overlap.
Section 8 Selection and Withdrawal of Subjects, Page 21	The study will enroll ~~at least 15 subjects/30 eyes~~ with CHM…	The study will enroll approximately 60 subjects with CHM, for a total of 120 treated eyes…	Clarified number of subjects and eyes enrolled in study
	~~At~~ the Screening ~~Visit~~, subjects ~~are~~ eligible…	During the Screening Period, subjects will be found eligible…	Clarified text
Section 8.1 Inclusion Criteria Page 21	~~≥34 ETDRS letters (equivalent to better than or equal 20/40 or better Snellen acuity) and ≤≥74 73 ETDRS letters (equivalent to better than or equal to 6/9 or 20/32 Snellen, decimal 0.63, LogMar 0.2) in both eyes1 or both untreated eyes*, if no prior treatment with AAV2-REP1 o≥74 ETDRS letters (equivalent to better than or equal to 6/9 or 20/32 Snellen, decimal 0.63, LogMar 0.2) in the untreated eye, if prior treatment with AAV2-REP1 was received in an alternative study*~~	3.Have documentation of a genetically-confirmed diagnosis of CHM 5. Have a BCVA of ≥34 ETDRS letters (20/200 or better Snellen acuity) in both eyes, or in the untreated eye, if the other eye was previously treated with AAV2-REP1* * If previously treated with AAV2-REP1 in an antecedent study, subjects may be eligible for participation following Sponsor approval. 6. For subjects who received treatment with AAV2-REP1 in an antecedent study, have biological samples available to complete an adequate immunology profile.	Updated inclusion criteria to study parameters, added criteria for subjects previously treated. See Section 7 in protocol for discussion of design and rationale for inclusion criteria.
Section 8.2 Exclusion Criteria Page 21	~~At~~ the Screening ~~Visit,~~ subjects ~~are~~ found not eligible…	During the Screening Period, subjects will be found not eligible…	Clarified text

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		2. Are unwilling to use barrier contraception methods or abstain from sexual intercourse for a period of 3 months following treatment with AAV2-REP1 in either eye.	Added text for clarity
Section 8.3 Subject Withdrawal Criteria Pages 22	~~Death~~		Removed text
	Severe ocular inflammation (e.g. endophthalmitis) that is unresponsive to treatment, ~~should result in the termination~~ of the ~~trial for the affected subject.~~ At the discretion of the investigator, if a persistent post-operative complication or poor visual function outcome presents post-operatively in SE1, the second eye may not be treated ~~and the subject may be discontinued from the study.~~	If severe ocular inflammation (e.g. endophthalmitis) or any other post-operative sequela that is unresponsive to treatment occurs in SE1 the second eye should not be treated. However, Period 1 should be continued for monitoring of safety for the duration of the study. At the discretion of the investigator, if a persistent post-operative complication or poor visual function outcome presents post-operatively in SE1, the second eye may not be treated. However, Period 1 should be continued for monitoring of safety for the duration of the study.	Updated/clarified other withdrawal criteria
		Subjects who withdraw from the study must undergo the Early Termination Visit assessments. See Table 1.	Added text for ET visit requirement.
Section 9.1 Treatments Administered, Page 23	At Period 1, Visit 1 (Day 0, the Injection Day Visit for SE1), subjects will ~~receive a volume of up to 0.1 mL of study drug containing 1×10^11 AAV2-REP1 gp. At Period 2, Visit 1 (Day 0, the Injection Day Visit for SE2), subjects will receive the same dose~~ via a sub-retinal injection.	At Period 1, Visit 1 (Day 0, the Injection Day Visit for SE1), subjects will receive in SE1 a sub-retinal injection up to 0.1 of study drug containing 1×10^11 AAV2-REP1 gp. At Period 2, Visit 1 (Day 0, the Injection Day Visit for SE2), subjects will receive in SE2 up to 0.1 mL of AAV2-REP1 via a sub-retinal injection.	Clarified text for treatments
Section 9.3 Packaging, Labeling, and Storage, Page 23	~~(on either the primary or secondary container) and include the protocol study number, Sponsor's name, product name, titre, vial and lot number, expiration date, storage conditions, and caution statement.~~	AAV2-REP1 is currently supplied in sterile single- use vials…	Clarified text
Section 9.4 Vitrectomy Procedure and		To improve visualization of the vector and facilitate dosing, surgeons are given the option of adding a minute quantity of trypan	Added text to include blue dye option for surgeons, clarified

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Injection of AAV2-REP1, Page 24		blue ophthalmic solution (~6 µL) to the vector solution. See the AAV2-REP1 Surgical Manual for further details. Prior to administration the vector needs to be primed… See the GEMINI Surgical Manual for further details.	text, added references.
Section 9.8 Concomitant Therapy, Page 25	Throughout the study, ~~investigators may~~ prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care. Details of concomitant medications will be collected ~~at the~~ Screening ~~Visit~~ and updated at every study visit (including the ET Visit, if applicable). Concomitant medications (including oral corticosteroid) taken during the study are to be recorded in the subject's medical records and eCRF; an exception to this is any medication used in the course of conducting a study ~~procedure~~ (e.g., anaesthesia, dilating eye drops). ~~To minimise inflammation resulting from surgery and potential or unexpected immune responses to vector/transgene, all subjects will be given a 21-day course of oral prednisone/prednisolone prior to each surgery2 .~~ ~~Administration of oral prednisone/prednisolone is to closely follow the 17-day protocol established in the Philadelphia AAV gene therapy clinical trial (Maguire 2008), except for the allowance of an extra 4~~	Throughout the study, subjects may be prescribed any concomitant medications, procedures, or treatments deemed necessary to provide adequate supportive care. Details of concomitant medications, procedures and/or treatments will be collected during the Screening Period and updated at every study visit (including the ET Visit, if applicable). Concomitant medications (including oral corticosteroid) taken during the study are to be recorded in the subject's medical records and eCRF; an exception to this is any medication used in the course of conducting study assessments (e.g., ophthalmic dyes, topical anesthesia, dilating eye drops). In addition, all subjects will be prescribed a course of oral corticosteroids. The oral prednisone / prednisolone regimen has been modified from the 17-day protocol established in the voretigene neparvovec-rzyl Philadelphia AAV gene therapy clinical trial for treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy (Maguire 2008), with the allowance of an extra 4 days for tapering the dose at the end of the course. The corticosteroid regimen is 1 mg/kg/day prednisone/prednisolone for a total of 10 days (beginning 2 days before the vector injection, on the day of injection, and then for 7 days); followed by 0.5 mg/kg/day for 7	This revision was made to include more than just concomitant medications, but also procedures or treatments, in the collection of medical status and updates of subjects. Also the details of the corticosteroid regimen are provided, and how these change depending on when the second eye is treated.

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~days for tapering the dose at the end of the course. Hence, for each surgery, this would be 1 mg/kg/day prednisone/prednisolone for a total of 10 days (beginning 2 days before vector injection, on the day of injection, and then for 7 days); followed by 0.5 mg/kg/day for 7 days; 0.25 mg/kg/day for 2 days; and 0.125 mg/kg/day for 2 days (21 days in total).~~ Details of corticosteroid usage will be captured by each subject in a diary card. ~~If the SE2 steroid treatment coincides with the SE1 steroid treatment period, an extended course of steroid will cover both surgical treatments~~.	days; 0.25 mg/kg/day for 2 days; and 0.125 mg/kg/day for 2 days (21 days in total; Study Day (SD) -2 through SD18). This regimen is outlined in detail below. Full Corticosteroid Regimen Prednisone or prednisolone administered as follows: □SD -2 through SD 7 (10 days): 1 mg/kg by mouth daily □SD 8 through SD 14 (7 days): 0.5 mg/kg/day by mouth daily □SD 15 through SD 16 (2 days): 0.25 mg/kg/day by mouth daily □SD 17 through SD 18 (2 days): 0.125 mg/kg/day by mouth daily For SE1 the Full Corticosteroid Regimen should be initiated as described. For SE2 surgeries that occur on day 21 or later, the Full Corticosteroid Regimen should be initiated as described, beginning 2 days before the scheduled SE2 surgery. If SE2 is to be performed prior to Study Day 21, then the Full Corticosteroid Regimen should be initiated 2 days prior to the scheduled surgery for SE2, and this will supercede the steroid taper in progress for SE1. The full 21 days of treatment will then be completed for SE2. If inflammation is observed in the study eye, and in the opinion of the investigator additional treatment with corticosteroid medication is indicated, corticosteroid therapy may be increased during the taper period (to a maximum of 1 mg/kg/day), may be reinitiated following completion of the taper, and/or may be supplemented by intraocular corticosteroids.	
Section 10.1 Screening Period	Screening ~~Visit~~ ~~(enzyme-linked immunosorbent assay [ELISA] and enzyme-~~	Screening Period	Updated Header, Removed text

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~linked immunospot [ELISPOT])~~		
		• Concomitant medication, procedures, and treatment review	Added text
	~~Subjects will be given two 21-day courses of oral corticosteroid (e.g., prednisolone / prednisone) and~~ instructed to start taking the drug days before their ~~next~~ surgery, at Period 1, Visit 1 for SE1 ~~and Period 2, Visit 1 for SE2.~~ Subjects will be issued a diary card to capture corticosteroid compliance ~~throughout each 21-day period~~. Subjects will also be instructed to use barrier contraception for a period of 3 months from the time they are treated.	For each subject, the interval between SE1 and SE2 treatment is expected to range from weeks to months. While this interval will be decided on a case-by-case basis, an effort will be made to schedule varying treatment intervals in order to better characterise the immunological and safety profile of sequential treatment administration and to reflect the anticipated real-world application of the study drug. Thus, approximately 20 subjects will be treated with a short- (<6 month), medium- (6-12 months), or long- (>12 month) duration surgery window between treatment of the first and second eye. Because the timing of the second surgery visit (Period 2, Visit 1) will vary among subjects, the duration of study Period 1 will also be variable. The next study visit (i.e., surgery and dosing, Period 1, Visit 1) is to be scheduled within 10 weeks of the Screening Period. Subjects will be treated with oral corticosteroids (e.g., prednisolone / prednisone) during the peri-operative period for each eye. They will be instructed to start taking the drug 2 days before their surgery at Period 1, Visit 1 for SE1. For SE1, the Full Corticosteroid Regimen should be initiated, as described in Section 9.8. For SE2 surgeries that occur on Day 21 or later, the Full Corticosteroid Regimen should be initiated as described, beginning 2 days before the scheduled surgery. If SE2 is to be performed prior to Study Day 21, then the Full Corticosteroid Regimen should be initiated 2 days prior to the scheduled surgery for SE2, and this	Updated screening visit to include discussion on subject duration of surgery window, corticosteroid treatment and regimen and baseline assessments.

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		will supercede the steroid taper in progress for SE1. The full 21 days of treatment will then be completed for SE2. Subjects will be issued a diary card to capture corticosteroid compliance. Subjects will also be instructed to use barrier contraception or abstain from sexual intercourse for a period of 3 months from the time they are treated. All baseline ophthalmic assessments must be conducted within 3 months prior to SE2 surgery (Period 2, Visit 1). If, at that time, BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the investigator and after consultation with the sponsor: 1. the subject is found eligible by all other inclusion and exclusion criteria, and 2. the subject is considered an appropriate candidate who will potentially benefit from treatment.	
Section 10.1.1 Baseline Visit, Period 2 (<12 Weeks from Visit 1, Period 2 [SE2]), Page 29		**10.1.1 Baseline Visit, Period 2 (<12 Weeks from Visit 1, Period 2 [SE2])** The following baseline assessments for Period 2 must be performed within 12 weeks prior to surgery for SE2 (Visit 1, Period 2). If the assessments have not been performed within a regularly scheduled visit within this window, an additional visit is required. • Demography, medical and ocular history update • Vital signs • Weight • Vector shedding sampling – blood, tears (both eyes), urine, saliva • Immunogenicity sampling • BCVA • Microperimetry • Full ophthalmic examination, including IOP, a slit-lamp examination, lens opacity, and dilated fundus examination • SD-OCT	Added section 10.1.1 for Baseline Assessments. This is a new visit that might be necessary for subjects whose second eye is treated an extended period of time from the first eye. This assures continued eligibility and safety of the subject for re-treatment.

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		• AF • 7-field colour fundus photography (including stereo photographs for fields 1, 2, and 3) • AE) and SAE monitoring • Concomitant medication, procedures and treatment review If BCVA in SE2 is <34 ETDRS letters, surgery may be performed when, in the opinion of the investigator and after consultation with the sponsor: 1. the subject is found eligible by all other inclusion and exclusion criteria, and 2. the subject is considered an appropriate candidate who has potentially modifiable disease.	
Section 10.2 Period 1 and 2, Visit 1 (Day 0, Injection Day Visit), Page 30		• Concomitant medication, procedures, and treatment review (including review of the corticosteroid diary card)	Updated text
	Subjects will then undergo vitrectomy and receive a volume to 0.1 mL sub-retinal injection of AAV2-REP1, containing 1 × 10^11 gp ~~in their first (SE1) eye~~	Subjects will then undergo vitrectomy and receive a volume of up to 0.1 mL sub-retinal injection of AAV2-REP1, containing 1 × 10^11 gp.	Clarified text
		or other post-operative sequelae	Added text
		If severe ocular inflammation or any other post-operative sequela that is unresponsive to treatment occurs in SE1, the second eye should not be treated. However, Period 1 should be continued for monitoring of safety for the duration of the study.	Added text for severe ocular inflammation or other post-operative sequela
Sections 10.3 – 10.9 Pages 31-33	~~(ELISA and ELISPOT)~~	At Visit X (Post -operative Day X), • Concomitant medication, procedures, and treatment review	Clarified text for post-operative Visits 3-8, and ET Visit in sections 10.3 – 10.9
Section 10.9 Early Termination (ET Visit), Page 31		10.9 Early Termination (ET Visit)	Added to header
Section 10.10 Unscheduled Visits, Page 34		(including review and return of the corticosteroid diary card)	Added text

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 11.1.1.1 Adverse Event, Page 35		Worsening of the signs, symptoms and visual function related to CHM is not to be considered an AE unless, in the opinion of the investigator, the changes are not consistent with the natural progression of the disease.	Text added to clarify that worsening of CHM is not considered an AE unless the PI considers it is inconsistent with the normal course of the disease.
	~~Surgery-related events of VA decrease are defined as VA decreases occurring in close temporal association (within <24 hours) with the surgical administration of the study medication, and which are resolving at Day 7 (Period 1/2, Visit 4) post-surgery. These events are not to be reported as an AE or SAE. However, they should be reported as an AE if in the investigator's opinion, their evolution in terms of duration or severity cannot be explained by the procedure. This would include, but not be limited to instances where the abnormal course of post-surgery VA decrease is associated with another complication attributable to the surgery or the study medication, or where the abnormal course of post-surgery VA decrease can be attributed to another identifiable cause.~~ ~~• AEs that in the opinion of the investigator, actually or potentially require any surgical or medical intervention to prevent permanent loss of sight.~~	**Vision Loss to be Reported as Serious Adverse Events** Vision Loss not to be Reported as an SAE: • Surgery-related BCVA decrease of ≥15 letters on ETDRS chart occurring within 1 day of surgery, but recovering / resolving at post-operative Days 7 and 14. Vision Loss or Vision-Threatening Event to be Reported as an SAE: • Surgery-related BCVA decrease of ≥15 letters on ETDRS chart that occurs within 1 day of surgery and that has not recovered* by the 1-Month Visit. • A decrease in BCVA of ≥15 letters on ETDRS chart that occurs within 1 day of surgery, however, in the investigator's opinion: ○ Has an evolution not consistent with the expected post-operative course; ○ May be attributable to a complication that occurred during surgery, or another untoward event, or the study drug; ○ Actually or potentially requires any surgical or medical intervention to prevent permanent loss of vision. • Non-surgery-related, sustained (>48 hours duration) decrease from baseline in BCVA of ≥15 letters on ETDRS chart. *Recovery / Resolution of BCVA is defined as a return to baseline BCVA within 5 letters on the ETDRS chart.	Clarified vision loss procedure for AE/SAE reporting. Without changing the meaning of the original language, we have revised this section to clarify what does and what does not need to be reported as an SAE regarding vision loss.

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 11.1.2 Recording of Adverse Events, Page 36	~~Non-serious AEs will be collected from Period 1, Visit 1 (Injection Day Visit for SE1) through Period 2, Visit 9 (or ET Visit, if applicable).~~	AEs/SAEs will be collected from the time the subject provides written informed consent (Screening Visit) through Period 2, Visit 9 (Month 12 ± 14 Days or ET Visit, if applicable).	Clarified text
	~~Unlikely to be Related: there are factors (evidence) explaining the occurrence of the event (e.g. progression of the underlying disease or concomitant medication more likely to be associated with the event) or a convincing alternative explanation for the event~~	AE severity and relationship to the study medication or the surgical procedure will be assessed at the site by the investigator or a medically qualified designee.	Added text to clarify AE severity and relationship assessments
		AE Severity **AE Relationship**	Added headers
		Related: A reasonable possibility exists that the study medication / study procedure caused the AE. A suspected AE can be further defined by: …	Added text to clarify suspected related AE definitions
	~~AE severity and relationship to the study medication or the surgical procedure will be assessed at the site by the investigator or a medically qualified designee.~~	When a relationship is determined to exist, the investigator or medical designee will further define if that relationship is to the study medication, the study procedure, both, or unknown.	Clarified text
Section 11.1.3 Follow-up of Adverse Events, Page 38	Investigators are expected to ~~timely~~ provide the requested additional information for a complete assessment and documentation of the SAE reports.	Investigators are expected to provide the requested additional information for a complete assessment and documentation of the SAE reports within a timely manner	Clarified text
Section 11.1.4 Reporting of Serious Adverse Events, Page 39	All cases that are fatal or life-threatening will be reported immediately, ~~and not later than 7 days…~~ …via phone ~~to the Chiltern Medical Monitor. The NSR Medical Monitor is the first escalation point when the Chiltern/NSR Medical Monitor is not available.~~	For reporting purposes, this time will be designated as Day 1. The Sponsor will report SAEs and Suspected, Unexpected, Serious Adverse Reactions (SUSARs) to investigative sites, the Institutional Review Board (IRB)s/Independent Ethics Committee (IEC)s, and regulatory authorities in compliance with current regulations. All cases	Updated the adverse event reporting, what defines Day 1, and when serious life-threatening events should be reported

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~The second escalation point is the NSR Chief Medical Officer.~~	that are fatal or life-threatening will be reported immediately after the Sponsor received the initial report from the Investigator. Emergency contact information is provided below. Any urgent queries relating to subject safety should be communicated immediately via phone using the contact information provided below. Additional contact information will be available in the Site Operations Manual.	
	~~[redacted], MD [redacted] Medical Monitor NightstaRx, Ltd Contract Research Organisation Chiltern Medical Monitor (out of hours) EU [redacted], MD Chiltern Ltd. Direct: [redacted] Mobile: [redacted] E-mail: [redacted]~~	Pharmacovigilance 5003 S. Miami Blvd, Suite 500 Durham, NC 27703 Email: APV.AENightstar@ashfieldpv.com +1 919 401 8003 Contract Research Organisation Covance Clinical and Periapproval Services, Ltd Osprey House Maidenhead Office Park, Westacott Way, Littlewick Green Maidenhead SL6 3QH, UK +44 (0) 1628 548000	Updated Contact information
Section 11.2 Pregnancy, Page 40		In addition, if possible, outcome of the pregnancy (birth or spontaneous abortion) fathered by the subject should be recorded and any incidents of congenital abnormality or birth defect should be reported.	Clarified text to align with informed consent
Section 11.3 Stopping Criteria, Page 40	• Severe ocular inflammation (e.g. endophthalmitis) that is unresponsive to treatment should result ~~in termination of the trial for~~ the affected subject.	• Severe ocular inflammation (e.g. endophthalmitis) that is unresponsive to treatment should result in the affected subject not being treated in the second eye. However, the subject will continue to be followed for safety for the duration of the study.	Clarified text so that it is clear that first eye should be followed until event has resolved even if the second eye will no longer be treated
		At the request of the Data Monitoring Committee, the trial may be stopped based on specific safety concerns (ie, SUSARs), not	Clarified text

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		defined in the Investigator's Brochure (Section 6.3). The study may be discontinued if the Sponsor deems it necessary for medical, safety, regulatory, business, or other reasons consistent with applicable laws or regulations.	
Section 11.4.2 Immunogenicity	~~All immunogenicity samples will be sent to a central laboratory for analysis.~~	Selected samples will be analysed. Remaining samples may be stored for up to 15 years or per local regulations.	Removed test stating that all samples will be analysed. Added text that selected samples will be analysed. Added text regarding the storing of samples.
Section 11.6 Best-Corrected Visual Acuity, Page 41		**11.6 Best-Corrected Visual Acuity** To evaluate changes in BCVA over the study period, BCVA will be assessed using the ETDRS VA chart and performed for both eyes at the times indicated in Table 1. The BCVA test should be performed prior to pupil dilation, and distance refraction should be carried out before BCVA is measured. Initially, letters are read at a distance of 4 metres from the chart. If <20 letters are read at 4 metres, testing at 1 metre should be performed. BCVA is to be reported as number of letters read correctly by the subject. For BCVA, assessors will be appropriately qualified for conducting the assessment. BCVA is also used for evaluations of efficacy. If a subject is not able to perform an assessment due to poor VA, it will be documented accordingly in the eCRF and will not be recorded as a protocol deviation.	Re-ordered assessment to appear first in safety and then linked the respective assessments repeated in efficacy
Section 11.7 Microperimetry,		11.7 Microperimetry	Re-ordered assessment to

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Pages 41		Microperimetry will be conducted on both eyes at the times indicated in Table 1. Microperimetry will be conducted by certified technicians to assess changes in retinal sensitivity within the macula. All microperimetry images will be sent by the sites to a Central Reading Centre (CRC) for review. For complete technical specifications for microperimetry, refer to the Study Operations Manual (which will include procedures from the CRC regarding how measurements are to be taken).	appear first in safety and then linked the respective assessments repeated in efficacy
Section 11.8 Fundus Autofluorescence, Page 48		**11.8 Fundus Autofluorescence** To assess changes in the area of viable retinal tissue, fundus autofluorescence will be performed on both eyes at the times indicated in Table 1. All AF images will be performed by certified technicians at the site after dilation of the subject's pupil and sent to a CRC for review; the CRC will enter the data into the EDC system. For complete technical specifications for AF, refer to the Study Operations Manual (which will include procedures from the CRC regarding how measurements are to be taken).	Re-ordered assessment to appear first in safety and then linked the respective assessments repeated in efficacy
Section 11.9 Spectral Domain Optical Coherence Tomography, Page 42		**11.9 Spectral Domain Optical Coherence Tomography** SD-OCT will be performed on both eyes at the times indicated in Table 1. SD-OCT measurements will be taken by certified technicians at the site after dilation of the subject's pupil. All OCT scans will be submitted by the sites to a CRC where the scans will be evaluated; the CRC will enter the data into the EDC system. SD OCT will be used to quantify integrity of the ellipsoid zone and reduction in the signal from the outer nuclear layer and choroid. In addition, foveal changes	Re-ordered assessment to appear first in safety and then linked the respective assessments repeated in efficacy

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		will be assessed. For complete technical specifications for SD-OCT, refer to the Study Operations Manual (which will include procedures from the CRC regarding how measurements are to be taken).	
Sections 11.10 & 11.11, Page 42	~~11.6~~ Full Ophthalmic Examination 11.7 7-Field Colour Fundus Photography	11.10 Full Ophthalmic Examination 11.11 7-Field Colour Fundus Photography	Header numbers renumbered to account for additional sections above.
Section 11.11 Field Colour Fundus Photography, Page 42	~~(which will include procedures from the CRC regarding how measurements are to be taken).~~		Text removed
Section 12 Assessment of Efficacy, Page 43	~~All efforts should be made to conduct the efficacy assessments; if a subject is not able to perform an assessment due to poor visual acuity (VA), it will be documented accordingly in the eCRF and will not be recorded as a protocol deviation.~~ ~~To evaluate changes in VA over the study period, BCVA will be assessed using the ETDRS VA chart and performed for both eyes at the times indicated in Table 1.~~ ~~The BCVA test should be performed prior to pupil dilation, and distance refraction should be carried out before BCVA is measured. Initially, letters are read at a distance of 4 metres from the chart. If <20 letters are read at 4 metres, testing at 1 metre should be performed.~~ ~~BCVA is to be reported as number of letters read correctly by the subject.~~ ~~For BCVA, assessors will be appropriately qualified~~	The following assessments are primary safety assessments that will also be considered secondary efficacy endpoints. Best-Corrected Visual Acuity See Section 11.6. See Section 11.7. See Section 11.8. See Section 11.9.	Removed text from sections 12.1 – 12.4 and added references to safety sections for procedure information, added best-corrected to VA section header

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~for conducting the assessment.~~ ~~To assess changes in the area of viable retinal tissue, fundus autofluorescence will be performed on both eyes at the times indicated in Table 1.~~ ~~All fundus autofluorescence images will be performed by certified technicians at the site after dilation of the subject's pupil and sent to a CRC for review; the CRC will enter the data into the EDC system. For complete technical specifications for autofluorescence, refer to the Study Operations Manual (which will include procedures from the CRC regarding how measurements are to be taken).~~ ~~SD-OCT will be performed on both eyes at the times indicated in Table 1.~~ ~~12.4 SD-OCT measurements will be taken by certified technicians at the site after dilation of the subject's pupil. All OCT scans will be submitted by the sites to a CRC where the scans will be evaluated; the CRC will enter the data into the EDC system. SD OCT will be used to quantify integrity of the ellipsoid zone and reduction in the signal from the outer nuclear layer and choroid. In addition, foveal changes will be assessed. For complete technical specifications for SD-OCT, refer to the Study Operations Manual (which~~		

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~will include procedures from the CRC regarding how measurements are to be taken).~~ ~~Microperimetry will be conducted on both eyes at the times indicated in Table 1.~~ ~~Microperimetry will be conducted by certified technicians to assess changes in retinal sensitivity within the macula. All microperimetry images will be sent by the sites to a CRC for review; the CRC will enter the data into the EDC system. For complete technical specifications for microperimetry, refer to the Study Operations Manual (which will include procedures from the CRC regarding how measurements are to be taken).~~		
Section 13 Statistacal Considerations, Page 45	~~Details of the statistical analyses will be described separately in the Statistical Analysis Plan.~~		Removed reference to SAP
Section 13.1 Sample Size, Page 45	~~At least 15 subjects 30 eyes~~ are planned to be enrolled in the study. ~~This sample size~~is considered sufficient	Approximately 60 subjects are planned to be enrolled in the study. A total of 120 treated eyes is considered sufficient …	Updated number of subjects and eyes
Section 13.2 Missing Data, Page 45		Management of dropout and missing observations will depend on their nature and frequency and will be discussed as part of the Data Review meeting before database lock.	Added text for procedure
Section 13.3 Analysis Sets, Page 45	The '~~Safety~~ Analysis Set will consist of all subjects who ~~receive study treatment (vitrectomy/AAV2-REP1) in the second (study) eye.~~ The ~~Safety~~ Subjects Analysis Set will be the primary population for	The "All Treated Subjects' Analysis Set will consist of all subjects who attend the 'Day of Surgery' visit of Period 1 (or, of Period 2, for subjects who received AAV2-REP1 treatment in an antecedent study). The All Treated Subjects Analysis Set will be the primary population	Updated text for All Treated Subjects analysis

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	demographics, baseline characteristics, and safety and efficacy analyses.	for demographics, baseline characteristics, and safety and efficacy analyses.	
Section 13.4 Descriptive Statistics and Conventions, Page 45	13.4 Descriptive Statistics and Conventions Continuous variables (including changes from Baseline) will be summarised over time using descriptive statistics (i.e., mean, standard deviation, 95% confidence interval [CI], median, ~~Q1, Q3, P05, P95~~, minimum, and maximum). ~~Data of subjects with no surgery in the second eye will be listed.~~	13.4 Descriptive Statistics and Conventions Detailed specifications of the planned analyses will be included in a separate Statistical Analysis Plan (SAP). Continuous variables (including changes from Baseline) will be summarised over time using descriptive statistics (i.e., mean, standard deviation, 95% confidence interval [CI], median, first and third quartiles,Q1, fifth and ninety-fifth percentiles, minimum, and maximum). Baseline is defined, for each eye and each period, as the last non-missing value prior to treatment of the respective period, in the respective eye.	Added to header, Added text for SAP, Added text to clarify baseline descriptive statistics
Section 13.5 Demographics and Baseline Characteristics, Page 45		Demographics will be summarised for all subjects combined. Age at the date of the Period 1 informed consent will be calculated. Baseline ocular characteristics will be summarised by eye and period.	Updated to include age at informed consent calculation
Section 13.6 Safety Analysis, Pages 45 & 46	Statistical tests will ~~not~~ be performed. ~~Alternatively, 95% 2-sided CIs will be calculated where appropriate.~~	No statistical tests will be performed. Safety assessments that are also considered efficacy assessments are presented in this section and not repeated in the efficacy section.	Updated text for no statistical test being performed
Section 13.6.1 Visual Acuity, Page 46	~~The proportion of subjects with a ≥5, ≥10 and ≥15 letter decrease from baseline in BCVA will be summarised by eye, period and visit.~~ ~~The proportion of subjects with a ≥10 and ≥15 letter improvement from baseline in BCVA will be summarised by eye, period and visit. Mean BCVA and mean change from Baseline in BCVA will be tabulated by eye, period and visit.~~	13.6.1 Visual Acuity See Section 13.7.1	Added text to reference VA safety analysis section in Statistical Considerations

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 13.6.2 Ophthalmic Examination, Page 46	~~Full Ophthalmic Examination IOP and change from Baseline in IOP will be summarised by visit and eye. Abnormal slit lamp examination findings and dilated ophthalmoscopy findings will be summarised by visit and eye. Lens opacity categories and shifts from baseline will be summarised by visit and eye.~~	13.6.2 Ophthalmic Examination IOP and change from Baseline in IOP will be summarised by eye, period and visit. Abnormal slit lamp examination findings and dilated ophthalmoscopy findings will be summarised by eye, period and visit. Lens opacity categories and shifts from baseline will be summarised by eye, period and visit.	Updated text for ophthalmic examination
Section 13.6.3 Spectral Domain Optical Coherence Tomography, Page 46	~~Spectral Domain Optical Coherence Tomography~~	See Section 13.7.2	Added reference to efficacy description for SD-OCT analysis
Section 13.6.4 Fundus Autofluorescence, Page 46		See Section 13.7.3	Added reference to efficacy description for AF analysis
Section 13.6.5 7-Field Colour Fundus Photography, Page 46	~~7-Field Colour Fundus Photography Categories of colour fundus photography findings will be summarised by visit and eye.~~	Categories of colour fundus photography findings will be summarised by eye, period and visit.	Updated text to clarify summaries
Section 13.6.6 Microperimetry, Page 46	~~Microperimetry~~	See Section 13.7.4	Added reference to efficacy description for microperimetry analysis
Section 13.6.7 Adverse Events, Page 46	~~Spectral Domain Optical Coherence Tomography Microperimetry Full Ophthalmic Examination IOP and change from Baseline in IOP will be summarised by visit and eye. Abnormal slit lamp examination findings and dilated ophthalmoscopy findings will be summarised by visit and eye. Lens opacity categories and shifts from baseline will be~~	AEs will be coded using the Medical Dictionary for Regulatory Affairs, version 21.0 or higher . Events will be summarised by system organ class and preferred term and eye. The number of eyes with an AE, as well as the number of events, will be summarised, by period and eye. Similar summaries will be produced for study drug/procedure-related AEs, AEs leading to discontinuation, and SAEs. AEs will also be summarised by maximum severity, relationship	Addeded text for AE coding and summaries

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~summarised by visit and eye.~~ ~~7 Field Colour Fundus Photography~~ ~~Categories of colour fundus photography findings will be summarised by visit and eye.~~	to study drug/procedure, and time to onset and resolution.	
Section 13.6.8 Immunogenicity and Vector Shedding, Page 47			Heading number changed to 13.6.8
Section 13.6.9 Vital Signs, Page 47	~~14.6.3Adverse Events~~ ~~AEs will be coded using the Medical Dictionary for Regulatory Affairs, version XX. The version of the dictionary current at the time of database lock will be used. Events will be summarised by system organ class and preferred term. The number of subjects and the number of eyes with an AE, as well as the number of events, will be summarised. Similar summaries will be produced for study drug/procedure-related AEs, AEs leading to discontinuation, and SAEs.~~ ~~AEs will also be summarised by maximum severity, relationship to study drug/procedure, and time to onset and resolution.~~ ~~14.6.42.1.1Full Ophthalmic Examination~~ ~~IOP and change from Baseline in IOP will be summarised by visit and eye. Abnormal slit lamp examination findings and dilated ophthalmoscopy findings will be summarised by visit and eye.~~ ~~Lens opacity categories and shifts from baseline will be~~	13.6.9 Vital Signs Vital signs will be summarised by period and visit.	Moved all deleted text to safety section, Added vital sign to safety analysis

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~summarised by visit and eye.~~ ~~14.6.52.1.17 Field Colour Fundus Photography~~ ~~Categories of colour fundus photography findings will be summarised by visit and eye.~~ ~~14.6.62.1.1Vital Signs~~ ~~Vital signs will be summarised in a descriptive manner.~~		
Section 13.7.1 Visual Acuity Page 48		13.7.1 Visual Acuity The proportion of subjects with a ≥10- and ≥15-letter improvement from baseline in BCVA will be summarised by eye, period and visit. Mean BCVA and mean change from Baseline in BCVA will be tabulated by eye, period and visit.	These descriptions were moved from safety to efficacy in the statistical section
Section 13.7.2 Spectral Domain Optical Coherence Tomography, Page 48	~~See Section 13.6.3.~~	13.7.2 Spectral Domain Optical Coherence Tomography Vitreo-macular Interface Disease, Intraretinal Hyper-Reflective Spots, Cystoid Macular Edema, Subretinal Fluid, Subretinal Hyper-reflective Material and Pigment Epithelial Detachment will be summarised by eye, period and visit, as well as shifts from baseline.	These descriptions were moved from safety to efficacy in the statistical section
Section 13.7.3 Fundus Autofluorescence, Page 48	~~See Section 13.6.4.~~	13.7.3 Fundus Autofluorescence Total Area of Preserved AF and Distance from Foveal Center to nearest Border of Preserved AF, and their change from baseline, will be summarized by eye, period and visit.	These descriptions were moved from safety to efficacy in the statistical section
Section 13.7.4 Microperimetry, Page 48	~~Formal statistical tests will not be performed.The proportion of subjects with a ≥10- and ≥15-letter improvement from baseline in BCVA will be summarised by eye and by visit.~~ ~~Mean BCVA and mean change from Baseline in BCVA will be tabulated by eye and by visit.~~	13.7.4 Microperimetry Fixation Location and Stability, and their shift from baseline, will be summarised by eye, period and visit. Bivariate Contour Ellipse Area and Mean Sensitivity, and their change from baseline, will be summarised by eye, period and visit. An analysis specific to the Sensitivity Points will be performed, the details of which will be described in the GEMINI SAP	These descriptions were moved from safety to efficacy in the statistical section

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 14 Informed Consent, Page 47	~~Independent Ethics Committee (~~IEC~~)~~ / ~~Institutional Review Board (~~IRB~~)~~		Clarified text
		Male subjects……	Added Male to clarify subject criteria
Section 14.3 Regulatory Considerations, Page 47		The study, where permissible, …	Clarified data
Section 15.2 Data Handling and Records Management, Page 48	database ~~(by KCT Data)~~. database ~~(by KCT Data)~~.		Deleted text
Section 15.3 Access to source Documentation and Subject Privacy, Page 49	The study will comply with the data protection laws which require data to be anonymised. ~~as soon as it is practical to do so.~~		Deleted text
Section 15.4 Time and Schedule of the Study, Page 49		The estimated overall study duration is 12 months of follow-up per eye.	Clarified text
Reference List, Page 50		Charles River Final Report Study Number 5700437; Sponsor Reference Number NSRP0914. A 4 and 24 week (single dose) ocular tolerance study following subretinal injection of a rAAV2.REP-1 vector in the Brown Norway rat. IND SN0000, Module 4.2.3.1. Streilein JW. Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. J Leukoc Biol 2003;74(2):179-85. Stein-Streilein J, Watte C. Cross-talk among cells promoting anterior chamber-associated immune deviation. Niederkorn JY, Kaplan HJ (eds): Immune Response and the Eye. Chem Immunol Allergy. Basel, Karger, 2007, vol 92: 115-130.	Added references
Section 17 Appendix,			Changes reflect changes in

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Table 1 Schedule of Study Procedures Page 53 & 54			assessments detailed above under individual visits, Abbreviations updated
	~~Immunogenicity sampling (ELISA and ELISPOT)~~ ~~ELISA=enzyme-linked immunosorbent assay; ELISPOT=enzyme-linked immunospot~~	a. Screening Visit/Period must be performed <10 weeks of Period 1, Visit 1. Selection of SE1 and SE2 will be determined by the investigator in collaboration with the subject. b. Baseline assessments must occur before the SE2 is dosed. These can occur at a regularly scheduled visit if that visit is within 12 weeks prior to surgery in SE2. If it has been more than 12 weeks since a visit, the subject must return for assessments at this Baseline Period 2 visit. Similarly, for subjects who have previously been treated unilaterally with AAV2-REP1 in an antecedent study, these baseline assessments must be performed ≤12 weeks prior to surgery on Visit 1, Period 2. A separate visit is only required if the assessments have not been performed within 12 weeks prior of surgery in SE2. c. Subjects will undergo vitrectomy and AAV2-REP1 administration to SE1 in Period 1; SE2 in Period 2. d. ET visit is to be performed if a subject discontinues at any time. e. If clinically indicated, subjects may need to return to the site for an unscheduled visit. At a minimum, the following assessments will be performed: BCVA, full ophthalmic examination, SD-OCT, autofluorescence, concomitant medication, procedures and treatment review and AE/SAE monitoring. f .Weight is collected for dose calculation of the corticosteroid regimen.	Updated text in all footnotes to reflect changes in schedule of assessments

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		g. Blood, tears (both eyes), urine and saliva samples. h. If ET visit occurs within 3 months post-treatment. i. Immunoassays are planned to assess cell-based and antibody-based immune responses against AAV2-REP1. Enzyme-linked immunospot (ELISPOT) assays will be used for the assessment of T-cell-mediated immune responses to transgene product (REP1) and the capsid. Antibody responses against the capsid and transgene product will be measured using a cell-based neutralization assay and an enzyme-linked immunosorbent assay (ELISA), respectively. All immunogenicity samples will be sent to a central laboratory for analysis. j. The ophthalmic examination will include IOP, slit lamp examination, lens opacity grading, and dilated ophthalmoscopy. The same slit lamp machine and lighting conditions should be used across the study for each subject. k. Stereo photos for fields 1, 2, 3. l. AEs/SAEs will be collected from the time the subject provides written informed consent (Screening Visit) through Period 2, Visit 9 (or ET Visit, if applicable). m. Subjects will be given a course of oral corticosteroid before each surgical visit, and instructed to start taking the drug 2 days prior to SE1/SE2 treatment, unless the SE2 surgical treatment coincides with the SE1 steroid treatment period, when an extended course of steroid can cover both surgical treatments. This regimen is outlined in detail in Section 9.8. For SE1, the Full Corticosteroid Regimen should be initiated as described. For SE2, and surgeries that occur on day 21 or later, the Full Corticosteroid Regimen should	

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		be initiated as described, beginning 2 days before the scheduled SE2 surgery. If SE2 is to be performed prior to Study Day 21, then the Full Corticosteroid Regimen should be initiated 2 days prior to the scheduled surgery for SE2, and this will supercede the steroid taper in progress for SE1. The full 21 days of treatment will then be completed for SE2	
Section 17 Table 1 Headers	~~Screening~~	Period 1 Screening[a] Period 2 Screening[a] Baseline[b]	Added column to separate Screening Visit to 2 columns;, Period 1 Screening & Period 2 Baseline, Clarified text in superscript to reference footnotes
	Day 0 Injection ~~Day[b]~~	Day 0 Injection Day[c]	
	ET ~~Visit[c]~~ Unscheduled ~~Visit[d]~~	ET Visit[d] Unscheduled Visit[e]	
Table 1, Screening Column		Period 1 Screening[a]	Added column to present Period 1 Screening assessment procedures
Table 1 Assessment/ Procedures Column		Weight[f] Vector shedding sampling[g] Immunogenicity sampling[i] Full ophthalmic exam[j] 7-field colour fundus photos[k] AE/SAE monitoring[l] Concomitant medication, procedures, and treatment review[m]	Updated superscript to footnote changes
	~~Weight[e]~~ Vector shedding ~~sampling[f]~~ ~~(ELISA and ELISPOT)~~ Full ophthalmic ~~exam[h]~~ 7-field colour fundus ~~photos[i]~~ AE/SAE ~~monitoring[j]~~ Concomitant ~~medication[k]~~	a- Screening Visit/Period must be performed … b- Baseline assessments must occur before the SE2 is dosed. These can occur at a regularly scheduled visit if that visit is within 12 weeks prior to surgery in SE2. If it has been more than 12 weeks since a visit, the subject must return for assessments at this Baseline Period 2 visit. Similarly, for subjects who have previously been treated unilaterally with AAV2-REP1 in an antecedent study, these baseline assessments must be performed ≤12 weeks prior to surgery on Visit 1, Period 2. A separate visit is only	Updated footnote references, added footnotes b and i, clarified text, removed non-serious AE collection from footnote l, Added SE1 &SE2 corticosteroid regimen paragraph to end of footnotes

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		required if the assessments have not been performed within 12 weeks prior of surgery in SE2. f- Weight is collected for dose calculation of the corticosteroid regimen. i- Immunoassays are planned to assess cell-based and antibody-based immune responses against AAV2-REP1. Enzyme-linked immunospot (ELISPOT) assays will be used for the assessment of T-cell-mediated immune responses to transgene product (REP1) and the capsid. Antibody responses against the capsid and transgene product will be measured using a cell-based neutralization assay and an enzyme-linked immunosorbent assay (ELISA), respectively. All immunogenicity samples will be sent to a central laboratory for analysis. j- The ophthalmic examination will include IOP, slit lamp examination, lens opacity grading, and dilated ophthalmoscopy. The same slit lamp machine and lighting conditions should be used across the study for each subject. l- AEs/SAEs will be collected from the time the subject provides written informed consent (Screening Visit) through Period 2, Visit 9 (or ET Visit, if applicable). m- Subjects will be given a course of oral corticosteroid before each surgical visit, and instructed to start taking the drug 2 days prior to SE1/SE2 treatment, unless the SE2 surgical treatment coincides with the SE1 steroid treatment period, when an extended course of steroid can cover both surgical treatments. This regimen is outlined in detail in Section 9.8. For SE1, the Full Corticosteroid Regimen should be initiated as described. For SE2, and surgeries that occur on day 21 or later, the	

Summary of Changes Protocol NSR-RPGR-02 Version 3.0, 4 Feb 2019			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		Full Corticosteroid Regimen should be initiated as described, beginning 2 days before the scheduled SE2 surgery. If SE2 is to be performed prior to Study Day 21, then the Full Corticosteroid Regimen should be initiated 2 days prior to the scheduled surgery for SE2, and this will supercede the steroid taper in progress for SE1. The full 21 days of treatment will then be completed for SE2.	
Table 1 Footnotes	~~1~~ AEs/SAEs will be collected from the time the subject provides written informed consent (Screening Visit) through Period 2, Visit 9 (or ET Visit, if applicable). ~~Non-serious AEs will be collected from Period 1, Visit 1 (Injection Day Visit for SE1) through Period 2, Visit 9 (or ET Visit, if applicable).~~		Updated the adverse event collection to all AEs and not just SAEs

SUMMARY OF CHANGES

CLINICAL STUDY PROTOCOL
NSR-REP-02

AAV2-REP1

An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)

GEMINI Study

INDICATION:	Choroideremia
STUDY PHASE:	2
EUDRACT NUMBER:	2017-002395-75
SPONSOR:	NightstaRx Ltd Wellcome Gibbs Building, 215 Euston Road London NW1 2BE, UK Telephone: +44 (0) 207 611 2077
Final Protocol Date:	4 Jul 2017
Amendment 1	17 May 2018

SUMMARY OF CHANGES SPONSOR APPROVAL PAGE

Clinical Study Protocol Number:	NSR-REP-02
Protocol Title:	An Open-Label Safety Study of Retinal Gene Therapy for Choroideremia with Bilateral, Sequential Administration of Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1)
Summary of Changes for Protocol:	Version 2.0 17 MAY 2018

Approved By:

The person listed below is authorised to sign the summary of changes on behalf of NightstaRx Ltd.

[redacted] Date: [redacted]

[redacted] MD

[redacted]

SUMMARY OF CHANGES
PROTOCOL NSR-REP-02
Version 2.0, 17 MAY 2018

Overview / Rationale

This protocol amendment has clarified the requirement for an evaluation by the investigator of the status of the first operated eye, regarding post-operative inflammation and visual function, and of the visual function and health overall of both eyes before scheduling the second eye surgery. It also specifies stopping criteria if there are signs of inflammation in the first eye. Immunogenicity testing is clarified. Other minor changes and contact information have been updated.

Page, Section	Previous Text (Deleted Text Shown by ~~Strikethrough~~)	Revised Text (Added Text Shown as Red)	Rationale
Page 4, Contact Information	[redacted]	[redacted]	Personnel change
Synopsis **Study Design**	~~The target interval between the surgical procedure in SE1 and SE2 will be determined at the Screening Visit.~~		The time interval between surgeries is more clearly explained in subsequent paragraph.
		The interval between SE1 and SE2 treatment is expected to vary for each subject from a few weeks to many months. While the interval will be decided on a case-by-case basis, an effort will be made to schedule varying treatment intervals in order to better characterise the immunological and safety profile of sequential treatment administration. Each site will work with the Sponsor and the assigned Contract Research Organisation (CRO) to ensure an even spread across the intervals. Given that the timing of the second surgery	New text to clarify time period between surgeries and requirement for an assessment of overall ocular health and function before scheduling of second eye.

SUMMARY OF CHANGES
PROTOCOL NSR-REP-02
Version 2.0, 17 MAY 2018

Overview / Rationale

This protocol amendment has clarified the requirement for an evaluation by the investigator of the status of the first operated eye, regarding post-operative inflammation and visual function, and of the visual function and health overall of both eyes before scheduling the second eye surgery. It also specifies stopping criteria if there are signs of inflammation in the first eye. Immunogenicity testing is clarified. Other minor changes and contact information have been updated.

Page, Section	Previous Text (Deleted Text Shown by ~~Strikethrough~~)	Revised Text (Added Text Shown as Red)	Rationale
		visit (Period 2, Visit 1) will vary between subjects, the duration of study Period 1 will also be variable. To avoid unnecessary study visits, every effort should be made by the investigator to schedule Period 2, Visit 1 to coincide with a planned Period 1 post-treatment visit. The post-operative outcome and visual function of SE1 will inform the scheduling of Period 2. Significant signs of post-operative inflammation, as judged by the investigator, should be resolved in SE1 for the subject to continue to Period 2. After comprehensive evaluation of all safety assessments and visual functional tests of SE1 and SE2, the Investigator will decide the appropriate time to schedule SE2, Visit 1.	
Page 14, Section 5.3, Study Rationale		A multi-centre, global Phase 3 study of a single subretinal administration of AAV2-REP1 for the treatment of CHM is ongoing.	Added test referring to STAR Phase 3 study ongoing.

SUMMARY OF CHANGES
PROTOCOL NSR-REP-02
Version 2.0, 17 MAY 2018

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This protocol amendment has clarified the requirement for an evaluation by the investigator of the status of the first operated eye, regarding post-operative inflammation and visual function, and of the visual function and health overall of both eyes before scheduling the second eye surgery. It also specifies stopping criteria if there are signs of inflammation in the first eye. Immunogenicity testing is clarified. Other minor changes and contact information have been updated.

Page, Section	Previous Text (Deleted Text Shown by ~~Strikethrough~~)	Revised Text (Added Text Shown as Red)	Rationale
Section 7.1 Overall Study Design, Page 16		The interval between SE1 and SE2 treatment is expected to vary for each subject from a few weeks to many months. While the interval will be decided on a case-by-case basis, an effort will be made to schedule varying treatment intervals in order to better characterise the immunological and safety profile of sequential treatment administration. Each site will work with the Sponsor and the assigned Contract Research Organisation (CRO) to ensure an even spread across the intervals. Given that the timing of the second surgery visit (Period 2, Visit 1) will vary between subjects, the duration of study Period 1 will also be variable. To avoid unnecessary study visits, every effort should be made by the investigator to schedule Period 2, Visit 1 to coincide with a planned Period 1 post-treatment visit. The post-operative outcome and visual function of SE1	New text to clarify time period between surgeries and requirement for an assessment of overall ocular health and function before scheduling of second eye.

SUMMARY OF CHANGES
PROTOCOL NSR-REP-02
Version 2.0, 17 MAY 2018

Overview / Rationale

This protocol amendment has clarified the requirement for an evaluation by the investigator of the status of the first operated eye, regarding post-operative inflammation and visual function, and of the visual function and health overall of both eyes before scheduling the second eye surgery. It also specifies stopping criteria if there are signs of inflammation in the first eye. Immunogenicity testing is clarified. Other minor changes and contact information have been updated.

Page, Section	Previous Text (Deleted Text Shown by ~~Strikethrough~~)	Revised Text (Added Text Shown as Red)	Rationale
		will inform the scheduling of Period 2. Significant signs of post-operative inflammation, as judged by the investigator, should be resolved in SE1 for the subject to continue to Period 2. After comprehensive evaluation of all safety assessments and visual functional tests of SE1 and SE2, the Investigator will decide the appropriate time to schedule SE2, Visit 1.	
Section 7.1 Overall Study Design, Page 17	~~For each subject, the interval between SE1 and SE2 treatment is expected to range from a few weeks to many months. While the interval will be decided on a case-by-case basis, an effort should be made to schedule varying treatment intervals (e.g. 1, 3 or 6+ months apart) in order to better characterise the immunological and safety profile of sequential treatment administration. Each site~~		This text has been moved up and altered slightly as above to clarify time period between surgeries and requirement for an assessment of overall ocular health and function before scheduling of second eye.

SUMMARY OF CHANGES
PROTOCOL NSR-REP-02
Version 2.0, 17 MAY 2018

Overview / Rationale

This protocol amendment has clarified the requirement for an evaluation by the investigator of the status of the first operated eye, regarding post-operative inflammation and visual function, and of the visual function and health overall of both eyes before scheduling the second eye surgery. It also specifies stopping criteria if there are signs of inflammation in the first eye. Immunogenicity testing is clarified. Other minor changes and contact information have been updated.

Page, Section	Previous Text (Deleted Text Shown by ~~Strikethrough~~)	Revised Text (Added Text Shown as Red)	Rationale
	~~will work with the assigned Contract Research Organisation to ensure an even spread across the intervals. Given that the timing of the second surgery visit (Period 2, Visit 1) will vary between subjects, the duration of study Period 1 will also be variable. To avoid unnecessary study visits, every effort should be made by the investigator to schedule Period 2, Visit 1 to coincide with a planned Period 1 post-treatment visit.~~		
Section 7.2 Discussion of Design, Page 18-19	~~To minimise inflammation resulting from potential immune responses to vector, including a secondary immune response following administration of AAV2-REP1 in the second eye (SE2), subjects will be given a course of oral corticosteroid (e.g.,~~	To minimise inflammation resulting from potential immune responses to vector, including treatment of the second eye (SE2), subjects will be given a 21-day course of oral corticosteroid before each surgical visit, and instructed to start taking the drug 2 days prior to SE1/SE2 treatment. If the SE2 steroid treatment coincides with the	Clarified the corticosteroid regimen, also when the two surgeries are planned close in time

SUMMARY OF CHANGES
PROTOCOL NSR-REP-02
Version 2.0, 17 MAY 2018

Overview / Rationale

This protocol amendment has clarified the requirement for an evaluation by the investigator of the status of the first operated eye, regarding post-operative inflammation and visual function, and of the visual function and health overall of both eyes before scheduling the second eye surgery. It also specifies stopping criteria if there are signs of inflammation in the first eye. Immunogenicity testing is clarified. Other minor changes and contact information have been updated.

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	~~prednisolone/prednisone) which will commence prior to each surgery~~	SE1 steroid treatment period, an extended course of steroid will cover both surgical treatments.	
Section 8.3 Subject Withdrawal Criteria		• Other (to be specified on the electronic case report form [eCRF]). • Severe ocular inflammation (e.g., endophthalmitis) that is unresponsive to treatment should result in the termination of the trial for the affected subject. • At the discretion of the investigator, if a persistent post-operative complication or poor visual function outcome presents post-operatively in SE1, the second eye may not be treated and the subject may be discontinued from the study.	Text now clarifies that if the post-operatively severe ocular inflammation is present, the subject will be terminated from the trial, and that any post-operative complication or poor visual outcome will prevent the second eye from continuing to surgery.
Section 8.3 Subject Withdrawal Criteria, Page 20	~~The study may be discontinued if the Sponsor deems it necessary for medical, safety, regulatory or other reasons consistent~~		This sentence has been moved to Section 11.3

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	~~with applicable laws or regulations.~~		
Setion 9.4 Vitrectomy Procedure	~~A single dose of AAV2-REP1 will be injected into the sub-retinal fluid through the same entry site.~~		This sentence is repeated in paragraph below
		Air fluid exchange during pars plana vitrectomy is not required for this surgical procedure. However, if an air fluid exchange is considered necessary, patients must be instructed to avoid air travel, travelling to high elevations or scuba diving until the air bubble has completely dissipated from the eye. It may take one week or more following injection for the air bubble to dissipate. A change in altitude while the air bubble is still present can result in irreversible vision loss. The dissipation of the air bubble should be verified through meticulous ophthalmic examination.	Details of the vitrectomy procedure have been added for clarification.
Section 9.8, Concomitant		If the SE2 steroid treatment coincides with the SE1 steroid treatment period, an extended	Added this sentence to clarify

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Therapy, Page 25		course of steroid will cover both surgical treatments.	corticosteroid regimen when surgeries are planned close in time.
Section 10.2 Period 1 and 2, Visit 1 (Day 0, Injection Day Visit) Page 26		The post-operative outcome and visual function of SE1 will inform the scheduling of Period 2. Significant signs of post-operative inflammation, as judged by the Investigator, should be resolved in SE1 for the subject to continue to Period 2. After a comprehensive evaluation of all safety assessments and visual functional tests of SE1 and SE2, the Investigator will decide the appropriate time to schedule SE2, Visit 1.	New text to clarify time period between surgeries and requirement for an assessment of overall ocular health and function before scheduling of second eye.
Section 11.1.4 Reporting of Serious Adverse Events Page 32		Emergency contact information is provided below. Any urgent queries relating to subject safety should be communicated immediately via phone to the Chiltern Medical Monitor. The NSR Medical Monitor is the first escalation point when the Chiltern/NSR Medical Monitor is not available. The	Emergency contact information has been added.

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		second escalation point is the NSR Chief Medical Officer. Contact Name and Title Telephone, Email [redacted], MD [redacted] Medical Monitor NightstaRx, Ltd Direct: [redacted] Mobile: [redacted] E mail: [redacted] Chiltern Medical Monitor (out-of-hours) EU [redacted], MD Chiltern Ltd. Direct: [redacted] Mobile: [redacted] E mail: [redacted] Chiltern Medical Monitor (out-of-hours) US [redacted], MD Chiltern Ltd. Direct: [redacted] Mobile: [redacted]	

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		E mail: [redacted]	
11.3 Stopping Criteria		11.3 Stopping Criteria • Severe ocular inflammation (e.g., endophthalmitis) that is unresponsive to treatment should result in termination of the trial for the affected subject. • At the request of the Data Monitoring Committee, the trial may be stopped based on specific safety concerns (ie, SUSARs), not defined in the Investigator's Brochure (Section 6.3). • The study may be discontinued if the Sponsor deems it necessary for medical, safety, regulatory or other reasons consistent with applicable laws or regulations.	New Section added for additional safety gating and stopping criteria
11.4.2 Immunogenicity		For the evaluation of immunogenicity, blood will be collected at the times indicated in Table 1. Immunoassays are planned to assess cell-based and antibody-based immune responses against AAV2-REP1. ELISPOT assays will	Immunogenicity testing has been specified.

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		be used for the assessment of T-cell- mediated immune responses to transgene product (REP1) and the capsid. Antibody responses against the capsid and transgene product will be measured using a cell-based neutralization assay and an ELISA, respectively. All immunogenicity samples will be sent to a central laboratory for analysis. Refer to the Study Operations Manual for details on the shipping and handling of samples.	
Section 13.7 Efficacy Analysis Page 38	Formal statistical tests will not be performed ~~and 95% CIs will be 2-sided.~~	Any additional categorical/continuous efficacy endpoints will be summarised using descriptive statistics, with 95% 2-sided CIs will be calculated where appropriate.	The text has been aligned with that of safety analyses.
Section 13.7 Efficacy Analysis Page 38	~~Any additional continuous efficacy endpoints will be analysed using descriptive statistics.~~		The text has been aligned with that of safety analyses.
Table of Procedures		k. Subjects will be given two 21-day courses of oral	Steroid therapy in the peri-

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and Visits Page 46 Footnote k		corticosteroid before each surgical visit, and instructed to start taking the drug 2 days prior to SE1/SE2 treatment, unless the SE2 surgical treatment coincides with the SE1 steroid treatment period when an extended course of steroid can cover both surgical treatments.	operative period is clarified for when surgeries are temporally close.