COVER PAGE

Official Title:	A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-Linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)
NCT Number:	NCT03116113
Document Date:	Protocol Version 11.0: 06 October 2020

CLINICAL STUDY PROTOCOL
NSR-RPGR-01 / NCT03116113

AAV8-RPGR

A DOSE ESCALATION (PHASE 1), AND DOSE EXPANSION (PHASE 2/3) CLINICAL TRIAL OF RETINAL GENE THERAPY FOR X-LINKED RETINITIS PIGMENTOSA USING AN ADENO-ASSOCIATED VIRAL VECTOR (AAV8) ENCODING RETINITIS PIGMENTOSA GTPASE REGULATOR (RPGR)

XIRIUS STUDY

INDICATION: X-linked retinitis pigmentosa

STUDY PHASE: 1/2/3

EUDRACT NUMBER: 2016-003852-60

SPONSOR: NightstaRx Ltd
2nd Floor
10 Midford Place
London W1T 5BJ UK

Telephone: +44 (0) 1628 501000

ORIGINAL PROTOCOL DATE:	27 Sep 2016
VERSION 2:	01 Dec 2016
VERSION 3:	26 May 2017
VERSION 4:	15 Dec 2017
VERSION 5:	16 Jan 2018
VERSION 6:	18 MAY 2018
VERSION 7:	16 Nov 2018
VERSION 7.1	10 Dec 2018 (Administrative Amendment)
VERSION 8.0	18 Dec 2018
VERSION 8.1	08 Mar 2019 (Administrative Amendment)
VERSION 9.0	14 Aug 2019
VERSION 10.0	10 Oct 2019
VERSION 11.0	1 Oct 2020

CONFIDENTIALITY STATEMENT

SPONSOR APPROVAL PAGE

Clinical Study Protocol Number: 274RP101 (NSR-RPGR-01)

Protocol Title: A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

Protocol Date: 1 Oct 2020

Approved By: [redacted], MD, PhD

The person listed above is authorized to sign the protocol on behalf of NightstaRx Ltd./ Biogen MA.

Signed: [redacted] Date: 6 October 2020

[redacted], MD, PhD

Biogen

INVESTIGATOR'S SIGNATURE PAGE

Clinical Study Protocol Number: 274RP101 (NSR-RPGR-01)

Protocol Title: A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

Protocol Date: 1 Oct 2020

I have read the Investigator's Brochure for AAV8-RPGR and I have read Protocol NSR-RPGR-01 and agree to conduct the study as outlined and in compliance with the Declaration of Helsinki, the International Conference on Harmonisation guideline for Good Clinical Practice, 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

CLINICAL RESEARCH ORGANISATION CONTACT INFORMATION

[redacted]

[redacted], UK

[redacted]

Additional contact information is available in the Site Operations Manual.

1. PROTOCOL SYNOPSIS

Name of Sponsor/Company: NightstaRx Ltd

Name of Test Product: AAV8-RPGR

Protocol Title: A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

Protocol Number: NSR-RPGR-01

Study centers: The study will be conducted at approximately 7 centers across the United Kingdom and United States of America

Study period (years):	**Phase of development:** Phase 1/2/3
Overall study duration: Part I: 24 months Part II:12 months	

Study Objective: The objective of the study is to evaluate the safety, tolerability, and efficacy of a single sub-retinal injection of AAV8-RPGR in subjects with X-linked retinitis pigmentosa (XLRP).

Endpoints

Part I

Primary Endpoint:

The primary safety endpoints are the incidence of dose-limiting toxicities (DLTs), and treatment-emergent adverse events (TEAEs) over a 24-month period.

Secondary and Exploratory Endpoints:

- Change from baseline in microperimetry at 1, 3, 6, 9, 12, 18 and 24 months
- Change from baseline in best-corrected visual acuity (BCVA) at 1, 3, 6, 9, 12, 18, and 24 months
- Change from baseline in spectral domain optical coherence tomography (SD-OCT) at 1, 3, 6, 9, 12, 18 and 24 months
- Change from baseline in fundus autofluorescence at 1, 3, 6, 12, 18 and 24 months
- Change from baseline in other anatomical and functional outcomes at 1, 3, 6, 9, 12, 18 and 24 months

Part II

Primary Efficacy Endpoint:

The primary efficacy endpoint is the proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci of the 10-2 grid assessed by Macular Integrity Assessment (MAIA) microperimetry at 12 months.

Safety Endpoint:

The safety endpoint is incidence of TEAEs over a 12-month period.

Secondary Endpoints:

- Proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of 16 central loci of the 10-2 grid assessed by MAIA microperimetry at 1, 2, 3, 6, and 9 months
- Change from baseline in retinal sensitivity at the central 16 loci assessed by MAIA microperimetry at 1, 2, 3, 6, 9, and 12 months
- Proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of 68 loci of the 10-2 grid assessed by MAIA microperimetry at 1, 2, 3, 6, 9, and 12 months

- Change from baseline in retinal sensitivity at 68 loci assessed by MAIA microperimetry at 1, 2, 3, 6, 9, and 12 months
- Change from baseline in best-corrected visual acuity (BCVA) at 1, 2, 3, 6, 9, and 12 months
- Change from baseline in visual field assessed by Octopus 900 perimeter at 3, 6, and 12 months

Exploratory Endpoints:

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

Study Design: This is a Phase 1/2/3, first-in-human, multi-center, dose-escalation interventional study of AAV8-RPGR in male subjects with genetically confirmed XLRP. Part I is a dose-escalation study; Part II is a dose-expansion study, with 2 doses (2.5 × 10^11 vector genomes (vg) [high dose], 5 × 10^10 vg [low dose]) selected from Part I based on a benefit/risk assessment, and a third untreated group to allow for a controlled comparison of efficacy and safety.

Part I consists of 11 visits over a 24-month evaluation period. Part II consists of 10 visits over a 12-month evaluation period. At the Screening / Baseline Visit, each subject will be assessed for eligibility of both eyes. Only 1 eye will be randomized (the "study eye"), and the other eye will be designated as the "fellow eye". Selection of the "study eye" will be made on clinical grounds prior to randomization and will generally be the worse eye affected.

At the Injection Day Visit (Visit 2, Day 0), subjects will undergo vitrectomy and iatrogenic retinal detachment as part of a sub-retinal injection procedure for administration of AAV8-RPGR in their study eye. Subjects in the untreated control group will receive study-visit telephone calls to monitor AEs/SAEs and review concomitant medications on Visit 2 (Day 0) and on Visits 3 and 4 (post-operative Days 1 and 7).

To minimise inflammation resulting from surgery and/or vector/transgene, in Part I, all subjects will be given a 21-day course of oral corticosteroid (e.g., prednisolone/prednisone) that will start 2 days before the planned date of surgery.

In Part II, all adult subjects will be given a 9-week course of oral corticosteroid starting 3 days before surgery: 21 days at 60 mg, followed by 6 weeks of tapering doses. The dose regimen is adjusted for pediatric subjects treated in Part II (see Section 8.8). Subjects may also be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), administered via a deep sub-Tenon approach.

Part I: Dose-Escalation Study

Dose escalation part of the study will use a 3+3 escalation scheme (Storer 1989) and will involve up to 6 AAV8-RPGR dose cohorts: 5 × 10^9 vg (Cohort 1), 1 × 10^10 vg (Cohort 2), 5 × 10^10 vg (Cohort 3), 1 × 10^11 vg (Cohort 4), 2.5 × 10^11 vg (Cohort 5), and 5 × 10^11 vg (Cohort 6). Each eligible subject will receive AAV8-RPGR in their study eye and be monitored for DLTs.

Three to 6 subjects are planned per dose cohort; however, the actual number of subjects enrolled into each cohort will depend on the toxicity observed. If no DLTs are observed in the first 3 subjects treated within a cohort, then escalation to the next dose cohort can proceed. If 1 DLT is reported within a 3-subject cohort, an additional 3 subjects will be treated at the same dose. If there are no further DLTs reported in the additional 3 subjects, then escalation to the next dose cohort can proceed. If ≥2 subjects within a cohort (3 or 6 subjects) have a DLT(s), then the maximum tolerated dose (MTD) will be identified as the previous (lower) dose. If ≥2 subjects with a DLT are

reported within Cohort 1 (3 or 6 subjects), then dosing will cease under this protocol and further investigation may occur following a protocol amendment.

An independent Data Monitoring Committee (DMC) will review safety data before confirming whether escalation to a higher dose level can occur. There is a potential for surgical complications resulting in safety events that meet the criteria for a DLT. In such cases, the DMC will make the final adjudication as to whether the event is a DLT. The DMC will review safety data for each cohort when at least 3 subjects have been dosed at a particular dose level. However, if 2 subjects within a cohort have a DLT(s), dosing will not proceed to subsequent subjects until safety data are reviewed by the DMC. For the purpose of making decisions regarding dose escalation, the DMC will review safety data collected for at least 4 weeks from each subject in the last dosed cohort. In addition, the DMC will review cumulative safety data collected from all previously dosed cohorts and take these findings into consideration when making decisions on dose escalation.

DLTs are defined as any of the following events considered to be related to AAV8-RPGR:

- Sustained decrease in BCVA of ≥30 letters on the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart compared to baseline; sustained is defined as lasting 48 hours or more until recovery, with recovery defined as visual acuity (VA) returning to within 10 letters of baseline VA. An exception is made for surgery-related events occurring in close temporal association (within <24 hours) of the surgery.
- Vitreous inflammation, vitritis (>Grade 3 using standardised Nussenblatt vitreous inflammation scale grading) (Nussenblatt, Palestine et al. 1985)
- Any clinically significant retinal damage observed (e.g., retinal atrophy) that is not directly attributed to complications of surgery
- Any clinically relevant suspected unexpected serious adverse reaction (as defined in the Investigator's Brochure), with the exception of vision loss or vision-threatening events (as defined in Section 11.2.1)

Although the visit will still be considered out-of-window, if subjects are unable to attend the 24-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so; this will be considered their 24-month visit. Delays will be identified as protocol deviations (PDs) on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

At study completion, Part I subjects will be invited to participate in a separate long-term follow-up study that will collect efficacy and safety data up to 5 years from surgery.

Part II: Dose Expansion

In Part II, approximately 30 subjects will be randomized 1:1:1 to a high-dose (2.5 × 10^11 vg), a low-dose (5 × 10^10 vg), or a third untreated group to allow for a controlled comparison of efficacy and safety. Study data will be collected for both eyes of each subject. Since treatment requires an invasive surgical procedure under general anesthesia, the sponsor, investigator and the subject will be unmasked to the study procedure (i.e., vitrectomy and sub-retinal injection), however within the treated groups, the sponsor, investigator and subject will be masked to the assigned dose level. To further minimise potential bias of the treated and non-treated eye evaluations, all subjective ophthalmic assessments at the Screening/Baseline Visit (Visit 1) and from Month 3 (Visit 6) onwards will be conducted by an assessor who is masked to treated eye and treated vs. untreated control group.

All Part II subjects will be followed for 12 months with the pre-specified visit schedule.

During Part II, a single administrative interim analysis (IA) may be conducted. If an IA is performed, the study will continue as planned in a masked fashion.

Although the visit will still be considered out-of-window, if subjects are unable to attend the 12-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. This will be considered their 12-month visit. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by

the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

At study completion, treated subjects in Part II will be invited to participate in a separate long-term follow-up study that will collect efficacy and safety data up to 5 years from surgery.

Number of subjects (planned): Overall, the study is expected to enroll approximately 48 subjects: 18 in Part I and up to approximately 30 in Part II.

Inclusion Criteria: Subjects are eligible for study participation if they meet all the following inclusion criteria.

Part I

1. Subject is willing and able to give informed consent for participation in the study
2. Are male, ≥18 years of age, and able to comply and adequately perform all study assessments
3. Have a genetically confirmed diagnosis of XLRP (with RPGR mutation)
4. Have active disease clinically visible within the macular region in both eyes and defined as follows:
   - ellipsoid zone (EZ) on SD-OCT measured at screening, must be within the nasal and temporal border of any B-scan, and not be visible on the most inferior and superior B-scan
5. BCVA in both eyes that meets the following criteria, based on the cohort level:
   - Cohort 1: better than or equal to light perception
   - Cohorts 2-3: BCVA of 34-73 ETDRS letters (equivalent to worse than or equal to 6/12 or 20/40 Snellen acuity, but better than or equal to 6/60 or 20/200 Snellen acuity).
   - Cohort 4-6: better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity)

Part II

1. Subject / parent / legal guardian (if applicable) is willing and able to provide informed consent/assent for participation in the study
2. Are male, ≥10 years of age, and able to comply and adequately perform all study assessments
3. Documentation of a pathogenic mutation in the RPGR gene
4. Have a BCVA in both eyes that meets the following criteria:
   - Better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity).
5. Mean total retinal sensitivity in the study eye as assessed by microperimetry ≥ 0.1 dB and ≤8 dB

Exclusion Criteria: Parts I and II

Subjects are not eligible for study participation if they meet any of the following exclusion criteria:

1. Have a history of amblyopia in either eye
2. Are unwilling to use barrier contraception methods (if applicable), or abstain from sexual intercourse, for a period of 3 months following treatment with AAV8-RPGR
3. Have any significant ocular or non-ocular disease/disorder which, in the opinion of the investigator, may put the subjects at risk because of participation in the study, may influence the results of the study, may influence the subject's ability to perform study

diagnostic tests, or impact the subject's ability to participate in the study. This would include, but is not limited to:

a. clinically significant cataract
b. contraindication to oral corticosteroid
c. unsuitability for retinal surgery

4. 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 (including but not limited to: Intelligent Retinal Implant System implantation, ciliary neurotrophic factor therapy, nerve growth factor therapy).

Test product, dosage, and mode of administration: Part I, Dose Escalation, Phase 1: All subjects will undergo vitrectomy and receive a single sub-retinal injection of AAV8-RPGR. Subjects will be assigned to 1 of the following AAV8-RPGR dose levels: 5 × 10^9 vg, 1 × 10^10 vg, 5 × 10^10 vg, 1 × 10^11 vg, 2.5 × 10^11 vg, or 5 × 10^11 vg.

Part II, Dose Expansion, Phase 2/3: Subjects will be assigned to 1 of the following: high-dose (2.5 × 10^11 vg), low-dose (5 × 10^10 vg), or an untreated control arm.

Reference therapy (Comparator), dosage, and mode of administration: In Part I, no reference therapies will be administered. In Part II, 2 doses of AAV8-RPGR will be compared: a high-dose (2.5 × 10^11 vg) and a low-dose (5 × 10^10 vg) to an untreated control arm.

Criteria for Evaluation:

Safety: The safety evaluation will be based on adverse event reporting (including DLTs); full ophthalmic examination (including indirect ophthalmoscopy, slit-lamp examination, intraocular pressure [IOP], anterior chamber and vitreous inflammation grading and lens opacities classification system III [LOCS III] cataract grading); fundus photography; vital signs; and laboratory assessments (including laboratory safety parameters, viral shedding and immunogenicity).

Any safety information collected as a result of the efficacy assessments (e.g., BCVA) will also be used in the overall safety evaluation, as appropriate.

Efficacy: The efficacy evaluation will be based on microperimetry (MAIA), BCVA, SD-OCT, fundus autofluorescence, visual fields (Octopus 900 specified in Part II only), contrast sensitivity, low luminance visual acuity (LLVA), [redacted]

Statistical Methodology:

In Part I, the safety analysis set will include all patients enrolled in the study who received AAV8-RPGR treatment.

All analyses in Part I will be performed using the Safety Analysis Set.

In Part II, the Safety Analysis Set will consist of all subjects who are randomized, under both the 2- and the 3-arm randomization schemes, and receive study treatment when randomized to active treatment, or attend Visit 2 (telephone call) when randomized to control (no treatment). The Safety Analysis Set will be used for safety analyses and subjects will be analyzed based on the actual treatment received. The efficacy summaries of Part II will be generated using the 'Intent-to-treat (ITT) Analysis Set'. The ITT Analysis Set will include all subjects randomised under the 3-arm randomisation scheme. Subjects will be analysed based on the treatment which they were randomised to.

In Part II, a single administrative IA may be conducted. A final analysis will be conducted after all subjects complete the Month 12 Visit (or are discontinued).

Summary statistics will be presented for both eyes (Study Eye and Fellow Eye). Continuous variables and their change from baseline will be summarized using descriptive statistics (number of subjects [n], mean, standard deviation, median, minimum, maximum, first and third quartiles, fifth and ninety-fifth percentiles). Confidence intervals (CI) of the mean and the mean change from baseline at each visit maybe be provided, where applicable. For categorical variables, the number

and proportion of subjects pertaining to each category will be presented over time, and the CI maybe presented where applicable.

The primary endpoint in Part II, i.e., the proportion of study eyes with ≥7 dB improvement of retinal sensitivity from baseline at ≥5 out of the 16 central loci, will be compared between treatment groups (high dose vs untreated; low dose vs untreated) using the Fisher Exact-Boschloo test with a Berger-Boos correction of beta=0.001 (Berger and Boos 1994). The primary hypothesis will be tested using Hochberg's (Hochberg 1988) step-up method with familywise error rate controlled at one-sided 0.10. The study will be declared positive if either or both doses achieve statistical significance. In addition, the difference in proportions between treatment groups will also be presented with its CI calculated by the Miettinen and Nurminen (Miettinen and Nurminen 1985) method.

AEs will be summarized by system organ class and preferred term. Both the number of eyes/subjects experiencing an AE and the number of events will be summarized. The serious adverse events (SAEs), severity, and relationship to study drug/procedure will be also summarized. A by-subject listing of AEs and DLTs will be provided.

2. TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

3. ABBREVIATIONS AND DEFINITIONS

Abbreviation or Term	Definition
AAV	adeno-associated virus
AAV8-RPGR	AAV8 virus particle encapsulating 3.46 kb cDNA for the coRPGR gene
AE	adverse event
BCVA	best-corrected visual acuity
BGH	bovine growth hormone
BSS	balanced salt solution
cDNA	complementary deoxyribonucleic acid
CI	confidence interval
coRPGR	codon optimised human cDNA encoding RPGR
CRC	Central Reading Centre
DLT	dose-limiting toxicity
DMC	Data Monitoring Committee
eCRF	electronic case report form
EDC	electronic data capture
EMA	European Medicines Agency
ERG	electroretinography
ET	early termination
EZ	ellipsoid zone
ETDRS	Early Treatment of Diabetic Retinopathy Study
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GTMP	gene therapy medicinal product
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IA	interim analysis
IOP	intraocular pressure
IRB	Institutional Review Board
ITT	intent to treat
LLVA	low luminance visual acuity
LOCS III	lens opacities classification system III
MAIA	macular integrity assessment
[redacted]	[redacted]
MTD	maximum tolerated dose
ORF15	open reading frame 15
PD	protocol deviation
RP	retinitis pigmentosa
RPE	retinal pigment epithelium
RPGR	retinitis pigmentosa GTPase regulator
Rpgr	murine homologue of human RPGR
SAE	serious adverse event
SAP	statistical analysis plan

Abbreviation or Term	Definition
SD-OCT	spectral domain optical coherence tomography
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
VA	visual acuity
[redacted]	[redacted]
vg	vector genomes
WT	wild-type
XLRP	X-linked retinitis pigmentosa

4. INTRODUCTION

4.1. X-linked Retinitis Pigmentosa

Retinitis pigmentosa (RP) is a group of hereditary degenerative retinal disorders with progressive loss of photoreceptors and accumulation of retinal pigment deposits (Hamel 2006). Typically, patients first experience defective dark adaptation (night blindness), followed by worsening of peripheral vision and progressive loss of central vision, often leading to legal or total blindness (Petrs-Silva and Linden 2014). RP is generally characterized by nyctalopia, constricted visual fields, bone spicule pigmentation of the fundus, photoreceptor cell dysfunction and abnormal, diminished or absent a- and b-waves on electroretinography (ERG) (Chang, Vaccarella et al. 2011). Fundoscopy generally appears normal until progression to mid-stage RP, whereby bone spicule-shaped pigment deposits in the mid-periphery, retinal atrophy with narrowing of vessels and moderate pallor of the optic nerve become apparent. With end-stage RP, widespread pigment deposits are evident, reaching into the macula.

X-linked RP (XLRP) is a very severe form of RP, resulting in rapid disease progression and severe retinal dysfunction. The worldwide prevalence of XLRP is approximately 1:30,000 to 1:40,000 (Tee, Smith et al. 2016). Patients with XLRP typically experience onset of night blindness in the first decade, followed by progressive restriction of visual field and loss of visual acuity (VA). Most patients are legally blind by the end of the fourth decade.

To date, mutations in the following 3 genes have been identified as causes of XLRP: *RP2*; *RP3*, also known as the RP GTPase regulator (*RPGR*) gene; and *OFD1* (a rare cause of XLRP) (Webb, Parfitt et al. 2012). Approximately 75% of cases of XLRP are due to *RPGR* mutations; the worldwide prevalence of RPGR-associated XLRP is approximately 1:40,000 to 1:53,000 (Pelletier, Jambou et al. 2007, Shu, McDowall et al. 2008). RPGR protein is localized to the photoreceptor-connecting cilium and to the corresponding structures (transition zone) in primary cilia. It is involved in different aspects of the ciliary gate, with trafficking, sorting of cargoes and quality control of membrane proteins (Ferrari, Di Iorio et al. 2011, Megaw, Soares et al. 2015, Tee, Smith et al. 2016). Loss of RPGR function in the retina causes the progressive loss of photoreceptors.

Visual loss associated with any retinal disorder is extremely debilitating and significantly impacts the patient's quality of life. XLRP is incurable and treatment is supportive at best. There are no currently marketed therapies available for modifying the disease. Possible treatments/strategies include light avoidance, neurotrophic factors, retinal implants, electronic prostheses, docosahexaenoic acid with or without vitamin A and gene therapy (MacLaren, Pearson et al. 2006, Talcott, Ratnam et al. 2011, Zrenner, Bartz-Schmidt et al. 2011, Barry, Dagnelie et al. 2012, Wen, Tao et al. 2012, West, Pearson et al. 2012, Zhou, Ni et al. 2012, Birch, Weleber et al. 2013, Hoffman, Hughbanks-Wheaton et al. 2014, Hughbanks-Wheaton, Birch et al. 2014, Tee, Smith et al. 2016).

A significant unmet medical need exists for new and effective therapies for XLRP, especially those designed to halt or significantly reduce the rate of progression.

4.2. Molecular Genetics of RPGR

The short arm of the X-chromosome harbors *RPGR* at Xp21.1. The *RPGR* gene has multiple isoforms arising from alternative splicing or post-translational modification (Yan, Swain et al. 1998, Kirschner, Rosenberg et al. 1999, Hong and Li 2002, Schmid, Glaus et al. 2010). These variants are expressed in different amounts, mainly in the lung, kidney, retina and

testes, suggesting tissue-specific splicing with specific functions in different tissues (Schmid, Glaus et al. 2010).

The 2 major *RPGR* isoforms are the constitutive variant encoded by exons 1-19 ($RPGR^{Ex1-19}$) and the $RPGR^{ORF15}$ isoform, which consists of exons 1-14 of $RPGR^{Ex1-19}$ followed by a unique C-terminal exon called open reading frame 15 (ORF15; (Tee, Smith et al. 2016)). This terminal is a mutational "hot spot", as it encodes an unusual repetitive sequence of 567 amino acids rich in glycine and glutamic acid residues (Vervoort, Lennon et al. 2000, Tee, Smith et al. 2016). The $RPGR^{ORF15}$ isoform is only expressed in the retina, with its protein product localized to the photoreceptor-connecting cilium (Vervoort, Lennon et al. 2000, Hong and Li 2002, Mavlyutov, Zhao et al. 2002, Hong, Pawlyk et al. 2003). Mutations in the $RPGR^{ORF15}$ isoform are responsible for approximately 60% of all cases of XLRP (Vervoort, Lennon et al. 2000, Bellingrath, Ochakovski et al. 2017). Over 350 RPGR variants have been discovered, with more than half occurring in the ORF15 exon, suggesting the importance of this isoform in the retina (Fokkema, den Dunnen et al. 2005).

For more information on the molecular genetics of RPGR, refer to the Investigator's Brochure.

4.3. Gene Therapy for Treatment of Retinal Diseases

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 2018). 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, compartmentalization and the presence of a fellow 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. 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 localized treatment without intravenous delivery, thus decreasing the chance of systemic absorption and toxicity. Finally, the effects of localized 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). AAVs remain in the nucleus as episomes with no integration into the human genome, thereby decreasing the risk of insertional oncogenesis. AAVs elicit a minimal immune response and allow for stable and long-term transgene expression in different retinal cells, including photoreceptors, retinal pigment epithelium (RPE) cells, ganglion and Muller cells (Vandenberghe, Bell et al. 2011, Day, Byrne et al. 2014).

Numerous early phase clinical trials using AAV vectors are currently underway for potential treatment of various retinal diseases, including wet age-related macular degeneration (ClinicalTrials.gov Identifier: NCT01024998, NCT01301443), autosomal recessive RP (NCT01482195), X-linked retinoschisis, Leber congenital amaurosis (NCT00749957, NCT00516477, NCT01208389, NCT00999609), Leber hereditary optic neuropathy

(NCT02064569, NCT02652767) Stargardt disease (NCT01367444), achromatopsia (NCT02599922), Usher syndrome (NCT01505062, NCT02065011) and choroideremia (NCT01461213) (Boye, Boye et al. 2013, MacLaren, Groppe et al. 2014, Edwards, Jolly et al. 2016).

4.4. Rationale for Clinical Development of AAV8-RPGR

There has been great interest in developing a GTMP to replace the defective RPGR gene in patients with XLRP. When designing a potential GTMP, it is essential to know that the transgene meets the packaging limit of the vector. Because the *RPGR* ORF15 (3.46 kb) is within the cloning capacity of standard AAV vectors, and allows space for a choice of promoters, an AAV vector is a good candidate for delivery of the *RPGR* transgene.

Given the instability of the ORF14/15 region of RPGR, development of a stable gene therapy construct has proved challenging (Deng, Dyka et al. 2015, Wu, Hiriyanna et al. 2015, Pawlyk, Bulgakov et al. 2016). A novel GTMP, AAV8-RPGR (AAV2/8-RK.coRPGR) has been developed featuring a codon-optimised coding sequence of $RPGR^{ORF15}$, which stabilizes the gene therapy construct and minimizes mutations. Recent studies have shown that the codon-optimised *RPGR (coRPGR)* coding sequence features higher expression-levels than wild-type (WT) *RPGR*. This results in greater sequence stability, provides an identical protein product, $RPGR^{ORF15}$, and promotes rod and cone expression (Fischer, McClements et al. 2017). In these studies, the pharmacodynamic effect of AAV8-RPGR was demonstrated in two well-characterized mouse models of XLRP, the transgenic $Rpgr^{-/y}$ model and the $C57BL/6J^{Rd9/Boc}$ model. The former is a knockout of the entire RPGR gene, whereas the latter (Rd9) has a naturally occurring mutation in the ORF15 region; both mice exhibit slow outer retinal degeneration. Treatment with 1.5×10^9 vector genomes (vg) AAV8-RPGR appeared to be effective in both animal models, as evidenced by an enhanced ERG response compared with relevant controls. Further, treatment of *C57BL/6JWT* mice with 1.5×10^9 vg AAV8-RPGR did not lead to toxic ocular effects (Fischer, McClements et al. 2017). Results from the sponsor's toxicity study also indicate that administration of the same vector (AAV8-RPGR) is well tolerated in male C57BL/6J mice at dose levels of 1×10^9 and 3.54×10^9 vg/eye; results from the sponsor's biodistribution study also support a lack of toxicity.

Proof-of-concept has also been demonstrated by other researchers in *Rpgr* null and *Rpgr* knockout mice using an AAV8 vector encoding $RPGR^{ORF15}$ (Wu, Hiriyanna et al. 2015, Pawlyk, Bulgakov et al. 2016). Sustained efficacy was demonstrated up to 18 months following a single sub-retinal injection in $Rpgr^{-/y}$ mice, with a dose of 1 × 10^9 vg showing the best outcome overall (Wu, Hiriyanna et al. 2015). However, most of the RPGR protein expressed in these experiments was truncated, which could have affected protein folding. No overt toxicity was observed in *Rpgr null* mice administered AAV8-RPGR at a lower dose of 2 × 10^9 vg (Pawlyk, Bulgakov et al. 2016).

In studies conducted with AAV-2/5 vectors encoding $RPGR^{ORF15}$ in two naturally occurring canine models (XLPRA1 deletion, XLPRA2 deletion), efficacy was demonstrated up to 3 years, depending on stage of disease (Beltran, Cideciyan et al. 2012, Beltran, Cideciyan et al. 2015). Photoreceptor expression of RPGR was observed after gene transfer, with effective preservation of photoreceptor structure and function. These studies lend to the body of evidence supporting the development of gene therapy for the treatment of XLRP due to $RPGR^{ORF15}$.

In an ocular study conducted in monkeys, transgene expression following the sub-retinal administration of various doses of AAV2 (4 animals [4 eyes/dose]: 10^10 and 10^11) and AAV8 (10 animals [5 eyes/dose]: 10^8, 10^9, 10^10, and 10^11) carrying a green fluorescent protein transgene correlated with surgical, clinical and immunological observations (Vandenberghe, Bell et al. 2011). Both AAV2 and AAV8 demonstrated efficient transduction of transgene in the RPE, with AAV8 being markedly better at targeting photoreceptors cells. Efficiency of RPE transduction by AAV8 at 10^9 vg was similar to that observed with AAV2 at a 10-fold higher dose. RPE transduction did not increase linearly at higher doses. Dose-related immune responses to capsid were observed with each vector, and the highest dose (10^11 vg) resulted in retinal inflammation and thinning. Some extra-ocular (lateral geniculate nuclei) distribution of vector and transgene was also observed with the highest dose. Dose thresholds were identified to effectively deliver gene product to target cells without toxicity. The authors concluded that use of AAV8 at intermediate doses (~10^10) is likely the best approach for gene therapy trials in humans.

There are very limited published data available with AAV8 dosing in humans for ophthalmological indications, although two small studies are ongoing. A Phase 1/2 clinical trial sponsored by the National Eye Institute is currently underway to evaluate the safety and tolerability of intravitreal AAV-RS1 vector (AAV8-scRS/IRBPhRS) gene transfer to the retina of patients with X-linked juvenile retinoschisis (ClinicalTrials.gov Identifier: NCT02317887) (doses unspecified). Another Phase 1/2 clinical trial sponsored by STZ eye trial is currently underway to evaluate the safety and tolerability of sub-retinal AAV-CNGA3 vector (rAAV8.hCNGA3) gene transfer to the retina of patients with CNGA3-linked achromatopsia (ClinicalTrials.gov Identifier: NCT02610582), using doses between 1 × 10^10 and 1 × 10^11 vg. Preliminary results from this latter study of subjects dosed with 1 × 10^10 vg demonstrate acceptable safety (Fischer, Wilhelm et al. 2016), as do higher doses of up to 1 × 10^11 vg.

Given these encouraging findings, NightstaRx is developing AAV8-RPGR as a potential GTMP for the treatment of XLRP due to mutations in RPGR. Replacing the deficient RPGR in XLRP patients with new and viable RPGR is expected to slow or stop retinal degeneration and may improve visual function.

5. STUDY OBJECTIVES AND ENDPOINTS

5.1. Objective

The objective of the study is to evaluate the safety, tolerability, and efficacy of a single sub-retinal injection of AAV8-RPGR in subjects with XLRP.

5.2. Endpoints

5.2.1. Part I

5.2.1.1. Primary Endpoint

The primary safety endpoints are the incidence of dose-limiting toxicities (DLTs), and treatment-emergent adverse events (TEAEs) over a 24-month period.

5.2.1.2. Secondary and Exploratory Endpoints:

- Change from baseline in microperimetry at 1, 3, 6, 9, 12, 18 and 24 months
- Change from baseline in best-corrected visual acuity (BCVA) at 1, 3, 6, 9, 12, 18, and 24 months
- Change from baseline in spectral domain optical coherence tomography (SD-OCT) at 1, 3, 6, 9, 12, 18 and 24 months
- Change from baseline in fundus autofluorescence at 1, 3, 6, 12, 18 and 24 months
- Change from baseline in other anatomical and functional outcomes at 1, 3, 6, 9, 12, 18 and 24 months

5.2.2. Part II

5.2.2.1. Primary Efficacy Endpoint

The primary efficacy endpoint is the proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci of the 10-2 grid assessed by Macular Integrity Assessment (MAIA) microperimetry at 12 months.

5.2.2.2. Safety Endpoint

The primary safety endpoint is the incidence of TEAEs over a 12-month period.

5.2.2.3. Secondary Endpoints

- Proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of 16 central loci of the 10-2 grid assessed by MAIA microperimetry at 1, 2, 3, 6, and 9 months
- Change from baseline in retinal sensitivity at the central 16 loci assessed by MAIA microperimetry at 1, 2, 3, 6, 9, and 12 months
- Proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of 68 loci of the 10-2 grid assessed by MAIA microperimetry at 1, 2, 3, 6, 9, and 12 months
- Change from baseline in retinal sensitivity at 68 loci assessed by MAIA microperimetry at 1, 2, 3, 6, 9, and 12 months

- Change from baseline in BCVA at 1, 2, 3, 6, 9, and 12 months
- Change from baseline in visual field assessed by Octopus 900 perimeter at, 3, 6, and 12 months

5.2.2.4. Exploratory Endpoints

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

6. INVESTIGATIONAL PLAN

6.1. Overall Study Design

This is a Phase 1/2/3, first-in-human, multi-center, dose-escalation interventional study of AAV8-RPGR in male subjects with genetically confirmed XLRP. The study will be conducted in two parts: Part I is a dose escalation study, Part II is a dose-expansion study, with 2 doses selected from Part I based on safety and efficacy, and a third untreated group to allow for a controlled comparison of efficacy and safety.

Part I will identify the maximum tolerated dose (MTD) using a dose-escalation scheme. Part II will expand 2 doses, allowing for a broader assessment of the safety and efficacy of AAV8-RPGR with a larger sample size, including approximately 30 subjects randomized 1:1:1 to a high-dose, a low-dose, or an untreated arm. Part I primarily evaluates safety, defined by incidence of DLTs and TEAEs over a 24-month period. Part II evaluates safety and efficacy, with inclusion of a primary efficacy endpoint, improvement from Baseline in microperimetry, evaluated at 12 months, and safety and secondary efficacy evaluated at 1-, 2-, 3-, 6-, 9-, and 12-months post-treatment.

Under version 6 of the protocol, Part II subjects were to be randomised into 2 active groups with a 2:1 allocation ratio (high dose [2.5 × 10^11 vg]: low dose [5 × 10^10 vg]). Any subject randomised under this version will be followed for 12 months with the ongoing visit schedule and the data will be analysed separately (i.e., included in the Safety Analysis Set but not in the Intent-to-Treat Set, See Section 12.3).

At study completion, all Part I subjects and treated subjects in Part II will be invited to participate in a long-term follow-up study that will collect efficacy and safety data up to 5 years from surgery.

Part I will consist of 11 visits over a 24-month evaluation period. Part II consists of 10 visits over a 12-month period. At the Screening/Baseline Visit, each subject will be assessed for eligibility of both eyes. Only 1 eye will receive treatment (the “study eye”), and the untreated eye will be designated as the “fellow eye.” Selection of the “study eye” will be made on clinical grounds and will generally be the worse eye affected. This will be discussed in detail and agreed with each subject as part of the informed consent process. Once a subject has been randomised, a change in “study eye” designation is not permitted.

At the Injection Day Visit (Visit 2, Day 0), subjects will undergo vitrectomy and iatrogenic retinal detachment as part of a sub-retinal injection procedure for administration of AAV8-RPGR in their study eye. To minimise inflammation resulting from surgery and/or vector/transgene, in Part I, all subjects will be given a 21-day course of oral corticosteroid (e.g., prednisolone/prednisone) that will start 2 days before the planned date of surgery (see Section 8.8 for details). In Part II, all subjects may be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), administered via a deep sub-Tenon approach. Subjects will also be given a 9-week course of oral corticosteroid starting 3 days before surgery: 21 days at 60 mg, followed by 6 weeks of tapering doses. The dose regimen is adjusted for pediatric subjects treated in Part II (see Section 8.8).

Subjects in the untreated control group will receive study-visit telephone calls to monitor AEs/SAEs and review concomitant medications on Visit 2 (Day 0) and on Visits 3 and 4 (post-operative Days 1 and 7).

Subjects will be assessed for safety and efficacy throughout the study as indicated in the Schedule of Study Procedures (see Section 16.1). The safety evaluation will be based on the

occurrence of adverse event (AE) reporting (including DLTs); full ophthalmic examination (including indirect ophthalmoscopy, slit-lamp examination, intraocular pressure [IOP], anterior chamber and vitreous inflammation grading and lens opacities classification system III [LOCS III] cataract grading); fundus photography; vital signs; and laboratory assessments (including laboratory safety parameters, viral shedding and immunogenicity).

The efficacy evaluation will be based on microperimetry, BCVA, SD-OCT, fundus autofluorescence, visual fields (Octopus 900 specified in Part II only), contrast sensitivity, low luminance visual acuity (LLVA), [redacted]. 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 surgery is performed, it should be carried out at least 4 weeks before their final visit.

A Part I subject is considered to have completed the study if he completes the Month 24 assessments. Although the visit will still be considered out-of-window, if Part I subjects are unable to attend their final 24-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. Delays will be identified as protocol deviations (PDs) on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

A Part II subject is considered to have completed the study if he completes the Month 12 assessments. Although the visit will still be considered out-of-window, if Part II subjects are unable to attend their final 12-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

During Part II, a single administrative interim analysis (IA) may be conducted. If this IA is performed, the study will continue as planned in a masked fashion.

The end of the study is the date the last subject completes his final-visit 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.

6.1.1. Dose-Limiting Toxicity

DLTs are defined as any of the following events considered to be related to AAV8-RPGR and should be reported to the sponsor:

- Sustained decrease in BCVA of ≥30 letters on the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart compared to baseline; sustained is defined as lasting 48 hours or more until recovery, with recovery defined as visual acuity (VA) returning to within 10 letters of baseline VA. An exception is made for surgery-related events occurring in close temporal association (within <24 hours) of the surgery.
- Vitreous inflammation, vitritis (>Grade 3 using standardised Nussenblatt vitreous inflammation scale grading) (Nussenblatt, Palestine et al. 1985)

- Any clinically significant retinal damage observed (e.g., retinal atrophy) that is not directly attributed to complications of surgery
- Any clinically relevant suspected unexpected serious adverse reaction (as defined in the Investigator's Brochure), with the exception of vision loss or vision-threatening events (as defined in Section 11.2.1.2)

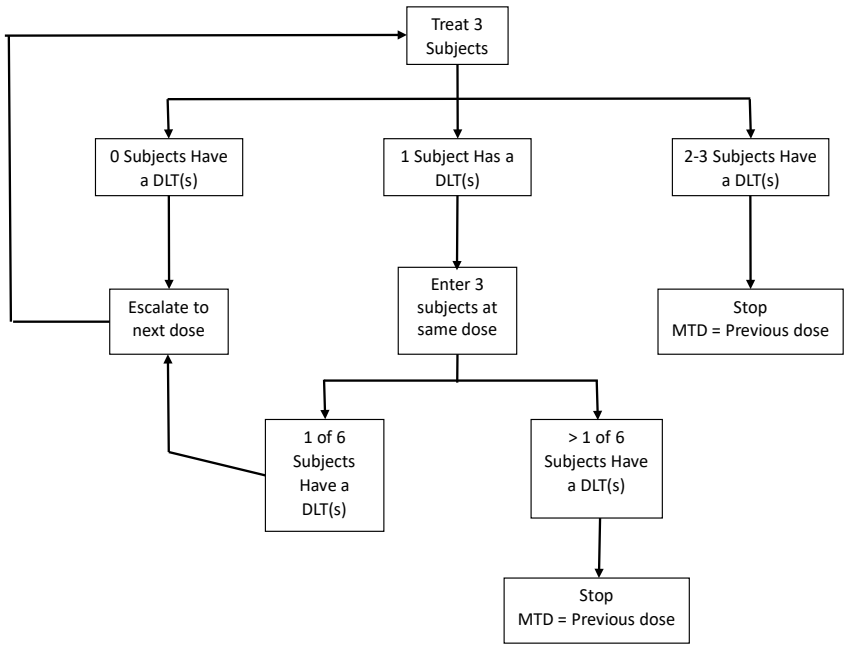
When triplicate BCVA assessments are performed at screening, the median BCVA result will be used for change-from-baseline BCVA computation.

If a DLT occurs at any time during the study, the site is to report the event to the sponsor within 24 hours of occurrence. A DLT will be reported by the investigational site, to the sponsor via a DLT/serious adverse event (SAE) reporting form that must be completed as fully as possible and emailed to [redacted] within 24 hours of being made aware of the event. Only 1 DLT/SAE form must be completed for each DLT.

6.1.2. Part I: Dose-Escalation Study

The study will use a 3+3 escalation scheme (Storer 1989) for administration of AAV8-RPGR; a schematic diagram of the escalation scheme is displayed in Figure 1.

Figure 1 3+3 Dose-Escalation Study



DLT: dose-limiting toxicity; MTD: maximum tolerated dose
Each dose level and potential DLTs will be reviewed by an independent Data Monitoring Committee. Data reviews will occur when at least 3 subjects have been dosed at a particular level or if/when 2 subjects have a DLT, whichever comes first. For making decisions regarding dose escalation, the DMC will review safety data collected for at least 4 weeks from each subject dosed at a particular level as well as cumulative safety data collected from previously dosed cohorts.

Part I of the study will involve up to 6 dose cohorts, with AAV8-RPGR doses of 5 × 10^9 vg (Cohort 1), 1 × 10^10 vg (Cohort 2), 5 × 10^10 vg (Cohort 3), and 1 x 10^11 vg (Cohort 4),

2.5 × 10^11 vg (Cohort 5), and 5 × 10^11 vg (Cohort 6). Each eligible subject will receive AAV8-RPGR in their study eye and will be monitored for DLTs.

An independent Data Monitoring Committee (DMC) will be used to review safety data before confirming whether escalation to a higher dose level can occur. There is a potential for surgical complications resulting in safety events that meet the criteria for a DLT. In such cases, the DMC will make the final adjudication as to whether the event is a DLT.

The DMC will review safety data for each cohort when at least 3 subjects have been dosed at a particular level. However, if 2 subjects within a cohort have a DLT(s), dosing will not proceed to subsequent subjects until safety data are reviewed by the DMC.

For making decisions regarding dose escalation, the DMC will review safety data collected for at least 4 weeks from each subject in the last dosed cohort. In addition, the DMC will review cumulative safety data collected from all previously dosed cohorts and take these findings into consideration when making decisions on dose escalation.

There will be a minimum of 4 weeks between each subject dosed in Cohort 1. Unless otherwise specified by the DMC, there will be no restrictions on the interval between subjects being dosed in Cohort 2 onwards.

Three to 6 subjects are planned per dose cohort; however, the actual number of subjects enrolled into each cohort will depend on the toxicity observed. If no DLTs are observed in the first 3 subjects treated within a cohort, then escalation to the next dose cohort can proceed. If 1 DLT is reported within a 3-subject cohort, an additional 3 subjects will be treated at the same dose. If there are no further DLTs reported in the additional 3 subjects, then escalation to the next dose cohort can proceed. If ≥2 subjects within a cohort (3 or 6 subjects) have a DLT(s), then the maximum tolerated dose (MTD) will be identified as the previous (lower) dose. If ≥2 subjects with a DLT are reported within Cohort 1 (3 or 6 subjects), then dosing will cease under this protocol and further investigation may occur following a protocol amendment.

Although the visit will still be considered out-of-window, if Part I subjects are unable to attend their final 24-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. This will be considered their 24-month visit. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

6.1.3. Part II Dose-Expansion, Version 6

Under version 6 of the protocol, Part II subjects were to be randomised into 2 active groups with a 2:1 allocation ratio (high dose [2.5 × 10^11 vg]: low dose [5 × 10^10 vg]). Any subject randomised under this version will be followed for 12 months with the ongoing visit schedule and the data will be analysed separately (i.e., included in the Safety Analysis Set but not in the Intent to Treat Set, See Section 12.3).

Enrollment under Version 6.0 has been superseded by the 3-arm randomisation ongoing since Version 7.

6.1.4. Part II: Dose Expansion

From version 9.0 thereafter, approximately 30 additional subjects will be randomized in a 1:1:1 allocation ratio to a high-dose group (2.5 × 10^11 vg), a low-dose group (5 × 10^10 vg), and an untreated group.

Study data will be collected for both eyes of each subject. Since treatment requires an invasive surgical procedure under general anesthesia, the sponsor, investigator and the subject will be unmasked to the study procedure (i.e., vitrectomy and sub-retinal injection), however within the treated groups, the sponsor, investigator and subject will be masked to the assigned dose. To further minimise potential bias of the treated and non-treated eye evaluations, all subjective ophthalmic assessments at the Screening/Baseline Visit (Visit 1) and from Month 3 (Visit 6) onwards will be conducted by a masked assessor.

During Part II, a single administrative IA may be conducted. If this IA is performed, the study will continue as planned in a masked fashion. A final analysis will be conducted at the end of the study, i.e., after all subjects complete the study. The end of the study is defined as the date the last subject completes his final-visit 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. See Section 12.8 for further details.

Although the visit will still be considered out-of-window, if Part II subjects are unable to attend their final 12-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. This will be considered their 12-month visit. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

6.2. Number of Subjects

Overall, the study is expected to enroll approximately 48 subjects: 18 in Part I and approximately 30 in Part II under 1:1:1 randomization scheme. See Section 12.1 for rationale.

6.3. Discussion of Study Design and Dose Selection

Guidelines published by the EMA and Food and Drug Administration (FDA) on mitigating risks in first-in-human studies and use of gene therapy in clinical trials were used in the design of this study (EMA 1994, EMA 2007, FDA 2015, EMA 2016, EMA 2018). An independent DMC will be used to review safety data before any dose escalation decisions are made.

The subjects to be included in the study are representative of active XLRP disease and are being selected to optimize observance of meaningful change in the outcome measures. The planned sample size in Part I is consistent with a 3+3 escalation scheme. A prospective trial period of 24 months for Part I is considered to be a sufficient period of time to monitor for any AEs related to the vector and/or transgene/administration procedure.

The starting dose being used in this clinical study is 5 × 10^9 vg AAV8-RPGR. This dose is primarily based on human equivalent doses (calculated on the basis of vitreous volume) from the AAV8-RPGR 26-week single-dose toxicity and biodistribution studies conducted by the sponsor and the mouse studies conducted at the University of Oxford (Fischer, McClements et al. 2017). In the Fischer studies, treatment with 1.5 × 10^9 vg AAV8-RPGR did not lead to toxic ocular effects in *C57BL/6JWT*. Results from the sponsor's toxicity and biodistribution studies indicate that AAV8-RPGR is well tolerated in male C57BL/6J mice at dose levels of 1 × 10^9 vg/eye and 3.54 × 10^9 vg/eye. The NOAEL was determined to be 3.54 × 10^9 vg/eye in mice, providing a 700-fold safety margin compared to the starting dose.

The second and third dose levels in this study are 1 × 10^10 and 5 × 10^10 vg. These dose increments are less than a 1-log increase from the previous dose levels (i.e., 5 × 10^9 and

1 × 10^10 vg respectively), considering the possibility of a narrow safe range for RPGR expression. Smaller dose increments were not expected to add meaningful information. Further, in a monkey study, dose thresholds of AAV8-GFP (an AAV8 virus particle encoding green fluorescence protein) were identified to effectively deliver gene product to target cells without toxicity, with the highest safe dose identified as 1 × 10^10 vg (Vandenberghe, Bell et al. 2011). In an ongoing Phase 1/2 clinical trial evaluating the safety and tolerability of sub-retinal AAV-CNGA3 vector (rAAV8.hCNGA3) in patients with CNGA3-linked achromatopsia, patients receive vector at doses between 1 × 10^10 and 1 × 10^11 vg (ClinicalTrials.gov Identifier: NCT02610582). Preliminary results from this clinical study in subjects dosed with 1 × 10^10 vg demonstrate acceptable safety (Fischer, Wilhelm et al. 2016), as do higher doses of up to 1 × 10^11vg.

The fourth (1×10^11 vg), fifth and sixth (2.5 × 10^11 and 5 × 10^11 vg) dose levels are less than a 0.5- log increase from the previous dose levels, ensuring a more conservative approach at the upper end of the dose-exploration range. The NOAEL in mice provides a 7-fold safety margin compared to the clinical maximum dose (5 × 10^11 vg).

Further details are provided in the Investigator's Brochure.Given previous experience demonstrating the safety of higher subretinal doses of AAV8 vector (Vandenberghe, Bell et al. 2011), and lacking safety signals at the lower range of doses, it should be possible to dose-escalate to the high end of the dose range of AAV8-RPGR.

Application of AAV8-RPGR to the under-surface of the retina requires retinal detachment following vitrectomy. As such, sub-retinal injection of AAV8-RPGR 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; 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 visual impairment. A long-term 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). To minimise inflammation resulting from potential immune responses to vector, subjects receiving AAV8-RPGR will be given a course of oral corticosteroid (see Section 8.8 for details).

Once Part I dose-escalation has been completed, and the safety and tolerability of AAV8-RPGR is demonstrated in adults, subjects ≥10 years of age will be enrolled in Part II of the study. The 10-years of age cut-off safeguards that participating pediatric subjects will be able to comply, adequately perform study assessments, and have sufficiently advanced disease that is encroaching on the macula (i.e., the AAV8-RPGR treatment administration area).

In Part II, subjects will be randomized to a high-dose (2.5 × 10^11 vg), a low-dose (5 × 10^10 vg), and an untreated group. This allows for comparisons in a randomized and controlled fashion, as recommended by regulators for best practices in ophthalmic gene therapy studies (FDA 2018). The sponsor, investigator and the subject will be unmasked to the study procedure and treatment (i.e. vitrectomy and sub-retinal injection). However, within the treated groups, the sponsor, investigator, and subject will be masked (i.e. double-masked) to the assigned dose. To further minimise potential bias of the treated and non-treated eye

evaluations, all subjective ophthalmic assessments at the Screening/Baseline Visit (Visit 1) and from Month 3 (Visit 6) onwards will be conducted by a masked assessor.

7. SELECTION AND WITHDRAWAL OF SUBJECTS

7.1. Part I

7.1.1. Inclusion Criteria

Subjects are eligible for study participation if they meet all the following inclusion criteria.

1. Subject is willing and able to provide informed consent for participation in the study
2. Are male, ≥18 years of age, and able to comply and adequately perform all study assessments
3. Have a genetically confirmed diagnosis of XLRP (with RPGR mutation)
4. Have active disease clinically visible within the macular region in both eyes and defined as follows:
    - ellipsoid zone (EZ) on SD-OCT measured at screening, must be within the nasal and temporal border of any B-scan, and not be visible on the most inferior and superior B-scan
5. Have a BCVA in both eyes that meets the following criteria, based on the cohort level:
    - Cohort 1: better than or equal to light perception
    - Cohorts 2-3: BCVA of 34-73 ETDRS letters (equivalent to worse than or equal to 6/12 or 20/40 Snellen acuity, but better than or equal to 6/60 or 20/200 Snellen acuity).
    - Cohort 4-6: better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity).

7.2. Part II

7.2.1. Inclusion Criteria

1. Subject / parent / legal guardian (if applicable) is willing and able to provide informed consent/assent for participation in the study
2. Are male, ≥10 years of age, and able to comply and adequately perform all study assessments
3. Documentation of a pathogenic mutation in the RPGR gene
4. Have a BCVA in both eyes that meets the following criteria:
    - Better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity).
5. Mean total retinal sensitivity in the study eye as assessed by microperimetry ≥0.1 dB and ≤8 dB

7.3. Exclusion Criteria: Parts I and II

Subjects are not eligible for study participation if they meet any of the following exclusion criteria:

1. Have a history of amblyopia in either eye

2. Are unwilling to use barrier contraception methods (if applicable), or abstain from sexual intercourse, for a period of 3 months following treatment with AAV8-RPGR
3. Have any other significant ocular or non-ocular disease/disorder which, in the opinion of the investigator, may put the subjects at risk because of participation in the study, may influence the results of the study, may influence the subject's ability to perform study diagnostic tests, or impact the subject's ability to participate in the study. This would include, but is not limited to, the following:
    a. clinically significant cataract
    b. contraindication to oral corticosteroid
    c. unsuitability for retinal surgery
4. 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 (including, but not limited to, Intelligent Retinal Implant System implantation, ciliary neurotrophic factor therapy, nerve growth factor therapy).

7.4. 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
- Death
- Other (to be specified on the electronic case report form [eCRF]).

If a subject discontinues the study, the reason for withdrawal is to be recorded in the eCRF. In the event that a subject discontinues the study early, the site should use every reasonable effort to ensure that an ET Visit is conducted as outlined in the Schedule of Study Procedures (see Section 16.1). If the subject is withdrawn due to an AE, the investigator will arrange for follow-up until the event has resolved or stabilized. For subjects who withdraw consent/assent, data will be collected through their last available study visit.

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

The study may be discontinued if the Sponsor deems it necessary for medical, safety, regulatory or other reasons consistent with applicable laws or regulations.

8. STUDY TREATMENT

8.1. Treatments Administered

Part I, Dose Escalation, Phase 1: all subjects will undergo vitrectomy and retinal detachment in their study eye and then receive a single, sub-retinal injection of AAV8-RPGR (See Section 8.4 for details). Subjects will receive an AAV8-RPGR dose of 5 × 10^9 vg (Cohort 1), 1 × 10^10 vg (Cohort 2), 5 × 10^10 vg (Cohort 3), 1 x 10^11 vg (Cohort 4), 2.5 × 10^11 vg (Cohort 5), or 5 x 10^11 vg (Cohort 6). See Section 6.1.2 for details.

Part II, Dose Expansion, Phase 2/3: Subjects will be assigned to 1 of the following: high-dose (2.5 × 10^11 vg), low-dose (5 × 10^10 vg), or an untreated control arm.

8.2. Description of Study Drug

The drug substance is the AAV8 vector containing recombinant human complementary deoxyribonucleic acid (cDNA) encoding RPGR (AAV8-RPGR). The vector genome (AAV8-coRPGR-BGH, known as AAV8-RPGR) is comprised of a strong constitutive expression cassette, a rhodopsin kinase promoter, the codon-optimised human cDNA encoding RPGR (coRPGR), and a bovine growth hormone (BGH)-polyA sequence flanked by AAV2 inverted terminal repeats. The codon-optimised human coding sequence of the retina-specific isoform $RPGR^{ORF15}$ was synthesized; the WT sequence of $RPGR^{ORF15}$ was also synthesized and provided in a pCMV6-XL vector backbone or in a pUC57 vector backbone for cloning.

The AAV8-RPGR 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% Pluronic F-68 (PF68). The drug product is a clear to slightly opalescent, colorless, sterile-filtered suspension with a target concentration of 5 × 10^12 vg/mL.

8.3. Packaging, Labeling, Preparation and Storage

AAV8-RPGR will be supplied in labelled sterile polypropylene tubes, with each tube containing 0.3 mL vector suspension. Thus, each tube will contain 1.5 × 10^12 vg in total.

AAV8-RPGR will be delivered in a total volume of up to 0.1 mL. Instructions for preparation and dilution of drug product to deliver the desired dose of AAV8-RPGR will be provided in the pharmacy manual.

Prior to shipment, each vial will be placed in a labelled secondary container. The drug product is to be stored at <−60°C (<−76°F) in a controlled access, temperature monitored freezer.

The Investigational Medicinal Product (IMP) will be labelled in compliance with regulatory standards.

8.4. Vitrectomy Procedure and Injection of AAV8-RPGR

The subretinal injection technique to be used in this study is similar to that developed in the sponsor's choroideremia programme in Oxford and other international investigator-sponsored trials in the United States, Canada and Germany. To date, over 200 subjects have been injected without complication by retinal surgeons using the technique described below.

All subjects may be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), administered via a deep sub-Tenon approach.

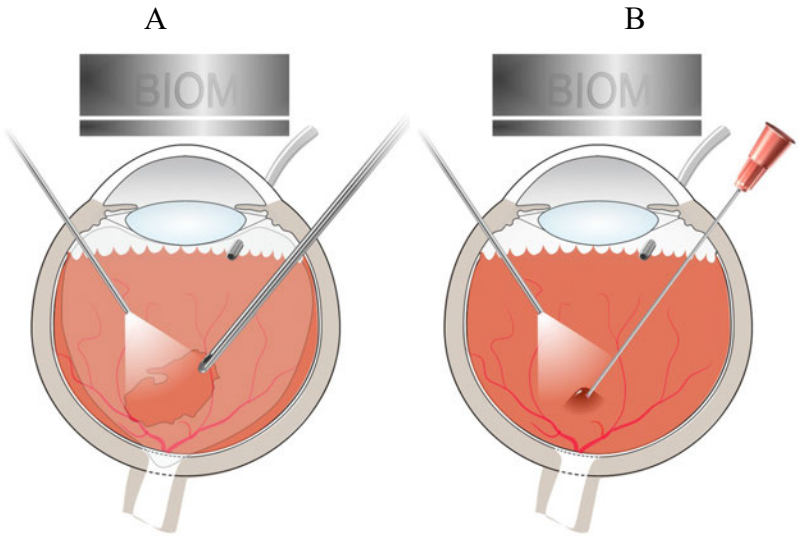
Injection of AAV8-RPGR is to be performed by an appropriately qualified and experienced retinal surgeon. All surgeons must have completed all study-specific surgical training and obtained certification by NightstaRx to perform the study procedure, before treating a study participant.

Initially, subjects will undergo a standard vitrectomy and detachment of the posterior hyaloid (Figure 2). All surgery 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. Prior to sub-retinal injection of AAV8-RPGR, 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. A single dose of AAV8-RPGR will be injected into the sub-retinal fluid through the same entry site. If detachment of the macula occurs with a smaller volume of fluid, then additional subretinal sites in the posterior globe (e.g., nasal to the disc) may also be chosen to deliver up to the entire 0.1 ml of vector. This avoids excessive foveal stretch.

If unexpected complications of retinal detachment are encountered (e.g., macular hole created requiring treatment with gas), the injection of vector can be deferred until a later date.

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.

Figure 2 Sub-retinal Injection of AAV8 Vector



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

8.5. Randomisation

Part I, the dose-escalation portion of this study, is open-label and not randomized.

In Part II, after the study eye is assigned, subjects will be randomised to 1 of 3 groups with a 1:1:1 allocation ratio: 1) treatment with AAV8-RPGR at a high dose (2.5 × 10^11 vg); 2) treatment with AAV8-RPGR at a low dose (5 × 10^10 vg) or 3) no treatment. Once a subject has been randomized, a change in "study eye" designation is not permitted. Randomisation will be generated using a validated system that automates the random assignment of treatment groups to randomisation numbers. Once a subject is deemed eligible, the investigative site (or authorized designee) will access the system, and the subject will be randomised using a standard blocked randomisation. The randomisation number will include the center number and subject number.

8.6. Study Masking

Part I of the study is open label.

Part II is double-masked (subject, surgeon, investigator/site team, and sponsor will be masked) to the assigned dose, and open-label with respect to the treatment procedure.

In Part II, ophthalmic assessments that will be used as efficacy endpoints (BCVA, LLVA, microperimetry, contrast sensitivity [redacted]) will be conducted by appropriately qualified assessors (see Table 1). For the immediate post-operative visits, masking of the assessors will not be viable as clinical signs of surgery will be apparent (i.e., redness, swelling). Therefore, unmasked assessors will perform all ophthalmic assessments at Visit 3 (Day 1), Visit 4 (Day 7), Visit 5 (Month 1), and Visit 5.9 (Month 2). From Visit 6 (Month 3) onwards, masked assessors will be used, as signs of surgery will have dissipated and it should not be possible clinically to differentiate between those subjects that have not undergone surgery, and those subjects that have undergone surgery and received active treatment. It is preferable that the same assessor who performed the efficacy endpoint assessments at Screening/Baseline also perform the assessments during the masked period from Month 3 to Month 12.

Table 1 Masked Assessments of Efficacy

Masked Assessments at Month 3, 6, 9 and 12 Post-Treatment with AAV8-RPGR
➢ Best-corrected visual acuity
➢ Low-luminance visual acuity
➢ Microperimetry
➢ Contrast sensitivity
➢ [redacted]

Subjects randomised to the untreated Control group will not be required to attend the site at Visit 2, 3 or 4. As the key purpose of Visit 2 is surgery, and Visit 3 and 4 are post-operative safety visits, there is limited utility in Control subjects attending. Therefore, to limit the study burden for Control subjects thereby potentially reducing the risk of subject withdrawal at this stage and reducing the possibility of further unmasking due to direct contact and communication with fellow participants, Control subjects are not scheduled to attend the clinic for study visits at these times.

To minimise bias further, masked assessors will not have access to the subject's medical records, source documentation or eCRF as data entries or notation (such as use of peri-

operative corticosteroid) may be sources of unmasking. From Visit 6 (Month 3) onwards, the masked assessor will also read a pre-written statement to each subject, regardless of randomisation, reminding them of the masked nature of the study, and to avoid any reference to prior surgery/non-surgery, which eye may have received treatment or to allude to any information that may unmask the assessor as to which group the subject has been assigned to.

Furthermore, it is anticipated that a subset of the subjects participating in the trial will be active on social media. Following appropriate approval by the Independent Ethics Committee (IEC)/Institutional Review Board (IRB), the patient information leaflet will request that subjects refrain from posting any details of study participation on social media, that may unmask the assessors to the group the subject has been assigned to. This request will be reiterated at subject visits by the investigator and within the pre-written statement.

Subjects randomised to the AAV8-RPGR treatment groups, surgeons, the investigative team, and the study sponsor will be masked to which dose of AAV8-RPGR the subject has been assigned to. Unmasked study site personnel will be assigned the responsibility of performing dilution, which will take place in a designated area remote from the investigative team to preserve masking of the treatment arm. Personnel delegated to perform the dilution will not be involved in any other aspect of the study (i.e., consent, safety/efficacy assessments, surgical procedure).

During Part II, a single administrative IA may be performed. If this IA is performed, the study will continue as planned in a masked fashion.

8.7. Study Drug Accountability

Records of the receipt and dispensing of study drug will be kept by each study center 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 centers will destroy all used vials in accordance with local procedures 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).

8.8. 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 (including, but not limited to, IRIS implantation, ciliary neurotrophic factor therapy, nerve growth factor therapy).

Throughout the study, subjects may be prescribed any concomitant medications, procedures and/ or treatments deemed necessary for the subjects' ongoing medical care. Details of medications, treatments and procedures will be collected at the Screening/Baseline Visit and updated at every study visit (including the ET Visit, if applicable). Concomitant medications (including prednisone/prednisolone), treatments and procedures taken/undergone 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 assessment (e.g., topical anesthesia, dilating eye drops).

To minimise inflammation resulting from surgery and potential or unexpected immune responses to vector/transgene, all subjects will be treated with a course of corticosteroid therapy.

In Part I, all subjects will be prescribed a 21-day course of oral prednisone/prednisolone following closely the 17-day protocol established in the voretigene neparvovec-rzyl AAV gene therapy clinical trial for treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy (Maguire, Simonelli et al. 2008), allowing an extra 4 days for tapering the dose at the end of the course, i.e., 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).

In Part II, all subjects will be prescribed a course of oral corticosteroids. In addition, at the time of surgery, subjects (adult and pediatric) may be treated with up to 1 mL of triamcinolone, 40 mg/mL solution, which must be administered via a deep sub-Tenon approach.

For adults, 60 mg of oral prednisone/prednisolone will be prescribed for the initial 21 days (starting 3 days prior to surgery), followed by a weekly taper as follows, for a total of 9 weeks of treatment:

Day -3 through day 17 (21 days): 60 mg by mouth once daily

Day 18 through day 24 (7 days): 50 mg by mouth once daily

Day 25 through day 31 (7 days): 40 mg by mouth once daily

Day 32 through day 38 (7 days): 30 mg by mouth once daily

Day 39 through day 45 (7 days): 20 mg by mouth once daily

Day 46 through day 52 (7 days): 10 mg by mouth once daily

Day 53 through day 59 (7 days): 5 mg by mouth once daily.

If at the Month-2 visit (Visit 5.9), inflammation is observed, corticosteroid therapy should be re-initiated, via oral and/or intraocular route, based on the clinical condition of the subject, and the judgement of the investigator.

For pediatric subjects, oral prednisolone/prednisone will also be started 3 days prior to surgery. The starting dose will be based on kilogram weight of the subject, up to a maximum of 60 mg starting dose (rounded to the nearest 1 mg). Subsequent doses will have multipliers to provide the appropriate taper over an additional 6 weeks, for a total of 9 weeks of treatment. See tapering regimen for pediatric subjects below:

Day -3 through day 17 (21 days): Starting Dose (SD) 1 mg/kg by mouth/ once daily (maximum dose of 60 mg once daily)

Day 18 through day 24 (7 days): SD X 0.83 mg by mouth once daily

Day 25 through day 31 (7 days): SD X 0.67 mg by mouth once daily

Day 32 through day 38 (7 days): SD X 0.5 mg by mouth once daily

Day 39 through day 45 (7 days): SD X 0.33 mg by mouth once daily

Day 46 through day 52 (7 days): SD X 0.17 mg by mouth once daily

Day 53 through day 59 (7 days): SD X 0.08 mg by mouth once daily

If at the Month-2 visit (Visit 5.9), inflammation is observed, corticosteroid therapy should be reinitiated, via oral and/or intraocular route, based on the clinical condition of the subject, and the judgement of the investigator.

The local pediatric team should be involved with all children undergoing gene therapy surgery and should be available to give advice on the steroid doses used in each patient. Modifications of the protocol-defined steroid treatment are allowed based on the recommendation of the pediatric team, with approval from the Sponsor.

For all subjects: while subjects are taking oral steroids, special note should be made to follow for potential side effects (e.g., increased IOP, cataracts, hypertension, elevated blood sugar, infections, gastritis/peptic ulcer disease, edema, electrolyte imbalance, mood changes, insomnia), and appropriate prophylaxis and/or therapy should be instituted as needed (e.g., treatment with proton pump inhibitors, valacyclovir; restriction of nonsteroidal anti-inflammatory agents).

8.9. Treatment Compliance

This study involves a single sub-retinal injection of up to 0.1 mL AAV8-RPGR. Measure of treatment compliance with AAV8-RPGR is therefore not necessary. The exact volume injected at the time of surgery should be recorded on the eCRF.

Compliance with the use of prednisone/prednisolone will be captured in the eCRF.

9. STUDY VISITS AND PROCEDURES

The schedule of study procedures is presented in Table 3, Section 16.1.

9.1. Visit 1 (Screening/Baseline 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 or parent / legal guardian will sign and date 1 copy of the consent form in the presence of the investigator or his/her designee; where applicable, an assent form will be completed by the subject. The original signed form(s) 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 or parent / legal guardian.

After informed consent/assent has been obtained, the subject will be evaluated to determine eligibility. Screening assessments will be considered baseline measurements and will consist of the following:

- Demography
- Medical history, including ocular history
- Blood pressure and pulse
- Collection of safety blood samples (hematology and clinical chemistry)
- RPGR gene mutation screen (only if not conducted previously)
- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and lens LOCS III cataract grading
- LLVA[1]
- ETDRS BCVA[1,2]
- SD-OCT
- Fundus autofluorescence
- MAIA microperimetry[1,3]
- Fundus photography
- Octopus 900 visual fields[3,4]
- Contrast sensitivity test[1]
- [redacted]
- Viral shedding
- Immunogenicity sampling
- AE and SAE monitoring
- Review of medication, treatments, and procedures
- [redacted][1,6,7]

- Randomisation[6]

  1. In Part II, it is preferable that the same masked assessor who performs these assessments (LLVA, BCVA, microperimetry, contrast sensitivity and [redacted]) during the masked period from Month 3 through Month 12 also perform these assessments at screening.

  2. For Part I subjects, BCVA is performed in triplicate at baseline. For Part II subjects, if the BCVA value at Visit 1 (Screening/Baseline) is ≥ ± 10 letter gain or loss in the study eye compared to the previous XOLARIS study visit (if applicable), then BCVA must be repeated an additional 2 times, resulting in a total of 3 BCVA measures at Visit 1.

  For Part II subjects, if the BCVA value at Visit 1 (Screening/Baseline) is < ± 10 letter difference in the study eye compared to the previous XOLARIS study visit, then BCVA will be collected once and will not be repeated.

  If subject was not previously in XOLARIS study, BCVA assessments at baseline must be performed in triplicate.

  For all subjects who require triplicate BCVA testing, to facilitate the additional BCVA measures, this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF. The highest score will be used to define subject eligibility.

  3. Assessments at screening collected in triplicate. To facilitate triplicate testing, the visit should be conducted over 2 days. Visual field and microperimetry outputs will be sent to a CRC for review. Data will be generated and collated within the CRC and exported to the Sponsor or designee. For microperimetry, the last assessment performed during screening will be used for assessing eligibility and will be defined as the baseline assessment.

  4. Octopus 900 perimeter is specified for visual field assessments only in Part II.

  [redacted]

  6. Part II only.

  [redacted]

Subjects who meet all the inclusion criteria and none of the exclusion criteria will have a study eye assigned and be enrolled into the study. In Part II, subjects will be informed of the randomisation outcome (i.e., AAV8-RPGR treatment or the Control group) and instructed to not reveal their treatment group assignment to the masked assessors during the study. Subjects randomised to the AAV8-RPGR treatment groups (along with the Investigators and sponsor) will remain masked to the assigned dose.

See Section 8.5 for details on randomisation and assignment of subject numbers.

The next study visit (Visit 2) is to be scheduled within 12 weeks of the Screening/Baseline Visit. Subjects will be given a course of oral prednisone/prednisolone and instructed to start taking the drug 2 days (Part I) or 3 days (Part II) before their next study visit (Visit 2). Where applicable, 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.

9.2. Visit 2 (Day 0, Surgery/Injection Day Visit)

At Visit 2, all subjects in the AAV8-RPGR groups will visit the surgical site, and the following assessments will be performed prior to surgery:

- Full ophthalmic examination, including indirect ophthalmoscopy, slit lamp examination with IOP assessment, anterior chamber, and vitreous inflammation grading, and LOCS III cataract grading

- Blood pressure and pulse
- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures
- Corticosteroid compliance review[1]

1. Corticosteroid review is applicable only for treated subjects.

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 analyses.

Subjects will then undergo vitrectomy and receive a sub-retinal injection of AAV8-RPGR (see Section 8.4 for details).

Subjects may be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), administered via a deep sub-Tenon approach.

Subjects will be carefully monitored for the occurrence of AEs during the procedure. Subjects may stay overnight or return to the site 1 day and then 7 days after surgery for post-operative follow-up (Visits 3 [Day 1] and 4 [Day 7], respectively).

Subjects in the Control group will receive a study visit Telephone Call at the agreed upon date and time. Sites will conduct the following assessments during the telephone call:

- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures

9.3. Visit 3 (Day 1 Post-Operative Visit)

At Visit 3, the first post-operative visit, the following assessments will be performed:

- Blood pressure and pulse
- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
- ETDRS BCVA
- SD-OCT
- Viral shedding
- Immunogenicity sampling
- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures
- Corticosteroid compliance review[1]

1. Corticosteroid review is applicable only for treated subjects.

Where applicable, subjects will be reminded of the requirement to use barrier contraception or abstain from sexual intercourse for a period of 3 months from the time of treatment.

Subjects in the Control group will receive a study visit Telephone Call at the agreed upon date and time. Sites will conduct the following assessments during the telephone call:

- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures.

9.4. Visit 4 (Day 7 Post-Operative Visit ± 3 Days)

At Visit 4, the second post-operative visit, the following assessments will be performed:

- Blood pressure and pulse
- Collection of safety blood samples (hematology and clinical chemistry)
- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
- ETDRS BCVA
- SD-OCT
- Viral shedding
- Immunogenicity sampling
- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures
- Corticosteroid compliance review[1]

1. Corticosteroid review is applicable only for treated subjects.

Where applicable, subjects will be reminded of the requirement to use barrier contraception or abstain from sexual intercourse for a period of 3 months from the time of treatment. Subjects in the Control group will receive a study visit Telephone Call at the agreed upon date and time (± 3 days of Day 7). Sites will conduct the following assessments during the telephone call:

- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures.

9.5. Visit 5 (Month 1 ± 7 Days)

At Visit 5, the following assessments will be performed:

- Collection of safety blood samples (hematology and clinical chemistry)
- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
- LLVA
- ETDRS BCVA
- SD-OCT
- Fundus autofluorescence
- MAIA microperimetry
- Viral shedding

- Immunogenicity sampling
- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures
- Corticosteroid compliance review[1]

1. Corticosteroid review is applicable only for treated subjects.

Where applicable, subjects will be reminded of the requirement to use barrier contraception or abstain from sexual intercourse for a period of 3 months from the time of treatment.

9.6. Visit 5.9 (Month 2 ± 7 Days)

At Visit 5.9, the following assessments will be performed:

- Collection of safety blood samples (hematology and clinical chemistry)
- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
- ETDRS BCVA
- SD-OCT
- Fundus autofluorescence
- MAIA microperimetry
- Viral shedding
- Immunogenicity sampling
- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures
- Corticosteroid compliance review[1]

1. Corticosteroid review is applicable only for treated subjects.

Where applicable, subjects will be reminded of the requirement to use barrier contraception or abstain from sexual intercourse for a period of 3 months from the time of treatment.

9.7. Visit 6 (Month 3 ± 7 Days)

At Visit 6 the following assessments will be performed:

- Collection of safety blood samples (hematology and clinical chemistry)
- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
- LLVA[1]
- ETDRS BCVA[1]
- SD-OCT
- Fundus autofluorescence

- MAIA microperimetry[1]
- Octopus 900 visual fields[2]
- Contrast sensitivity test
- Immunogenicity sampling
- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures
- [redacted][1,3]

1. In Part II, at Month 3, LLVA, BCVA, microperimetry and the [redacted] will be conducted by qualified masked assessors.

2. Octopus 900 perimeter is specified for visual field assessments only in Part II.

[redacted]

9.8. Visit 7 (Month 6 ± 14 Days)

At Visit 7, the following assessments will be performed:

- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
- LLVA[1]
- ETDRS BCVA[1]
- SD-OCT
- Fundus autofluorescence
- MAIA microperimetry[1]
- Octopus 900 visual fields[2]
- Contrast sensitivity test[1]
- [redacted][3]
- Immunogenicity sampling
- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures

1. In Part II, at Month 6, LLVA, BCVA, microperimetry and the [redacted] will be conducted by qualified masked assessors.

2. Octopus 900 perimeter is specified for visual field assessments only in Part II.

[redacted]

9.9. Visit 8 (Month 9 ± 14 Days)

At Visit 8, the following assessments will be performed:

- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
- LLVA[1]
- ETDRS BCVA[1]
- SD-OCT
- MAIA microperimetry[1]
- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures

1. In Part II, at Month 9, [redacted], BCVA, and microperimetry will be conducted by qualified masked assessors.

9.10. Visit 9 (Month 12 ± 14 Days)

At Visit 9, the following assessments will be performed:

- Collection of safety blood samples (hematology and clinical chemistry)
- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
- LLVA[1]
- ETDRS BCVA[1,2]
- SD-OCT
- Fundus autofluorescence
- MAIA microperimetry[1]
- Octopus 900 visual fields[3]
- Fundus photography
- Contrast sensitivity test[1]
- [redacted][4]
- Immunogenicity sampling
- AE/SAE monitoring
- [redacted][1,5]
- Review of concomitant medication, treatments, and procedures

1. In Part II, at Month 12, LLVA, BCVA, microperimetry, contrast sensitivity, and the [redacted] will be conducted by qualified masked assessors.

2. Part I subjects perform BCVA assessments in triplicate at Month 12. To facilitate the additional BCVA measures, this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF.

3. Octopus 900 perimeter is specified for visual field assessments only in Part II.

[redacted]

Subjects who develop cataracts should undergo cataract surgery if deemed clinically necessary; if surgery is performed, it should be carried out at least 4 weeks before Visit 9 (Month 12) or Visit 11 (Month 24) for Part II and Part I subjects, respectively.

Although the visit will still be considered out-of-window, if Part II subjects are unable to attend their final 12-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. This will be considered their 12-month visit. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

9.11. Visit 10 (Month 18 ± 14 Days) (Part I Subjects Only)

At Visits 10, the following ocular assessments will be performed:

- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
- LLVA
- ETDRS BCVA
- SD-OCT
- Fundus autofluorescence
- MAIA microperimetry
- Fundus photography
- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures

9.12. Visit 11 (Month 24 ± 14 Days) (Part I Subjects Only)

At Visits 11, the following ocular assessments will be performed:

- Collection of safety blood samples (hematology and clinical chemistry)
- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
- LLVA
- ETDRS BCVA[1]
- SD-OCT
- Fundus autofluorescence
- MAIA microperimetry

- Fundus photography
- Visual Fields
- Contrast sensitivity test
- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures

1. Part I subjects perform BCVA assessments in triplicate at Month 24. To facilitate the additional BCVA measures, this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF.

Although the visit will still be considered out-of-window, if Part I subjects are unable to attend their final 24-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. This will be considered their 24-month visit. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

9.13. Early Termination (ET) Visit

If 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:

- Collection of safety blood samples (hematology and clinical chemistry)
- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
- LLVA[1]
- ETDRS BCVA[1]
- SD-OCT
- Fundus autofluorescence
- MAIA microperimetry[1]
- Octopus 900 visual fields[2]
- Fundus photography
- Contrast sensitivity test[1]
- [redacted]
- Immunogenicity sampling
- AE/SAE monitoring
- [redacted][1,4]
- Review of concomitant medication, treatments, and procedures

1. In Part II, at the ET Visit, LLVA, BCVA, microperimetry, contrast sensitivity, and the [redacted] will be conducted by qualified masked assessors.

2. Octopus 900 perimetry is specified for visual field assessments only in Part II.

3. In Part II and only at sites with available technology.

[redacted]

9.14. 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.

- Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
- ETDRS BCVA[1]
- SD-OCT
- Fundus autofluorescence
- MAIA microperimetry, if clinically feasible[1]
- AE/SAE monitoring
- Review of concomitant medication, treatments, and procedures

1. For unscheduled visits that occur during Part II, BCVA and microperimetry will be conducted by qualified masked assessors from Month 3 through Month 12.

10. ASSESSMENT OF EFFICACY

All efforts should be made to conduct the efficacy assessments; 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.

10.1. Best-Corrected Visual Acuity

To evaluate changes in VA over the study period, BCVA will be assessed for both eyes using the ETDRS VA chart at the times indicated in Table 3, Section 16.1.

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 meters from the chart. If <20 letters are read at 4 meters, testing at 1 meter should be performed. BCVA is to be reported as number of letters read correctly by the subject.

In Part I, at the Screening/Baseline Visit, eyes will be eligible for the study if they:

- Have better than or equal to light perception (Cohort 1 only), or
- Have a BCVA of 34-73 ETDRS letters (equivalent to worse than or equal to 6/12 or 20/40 Snellen acuity, but better than or equal to 6/60 or 20/200 Snellen acuity) (Cohorts 2-3)
- Have a BCVA better than or equal to 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity) (Cohort 4 [5 and 6, if applicable] and MTD cohort)

In Part II, at the Screening/Baseline Visit, eyes will be eligible for the study if they have a BCVA better than or equal to 34 ETDRS letters.

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

For Part I subjects, BCVA will be performed in triplicate over a 2-day period at Visits 1, 9 and 11 (or ET Visit) for all subjects. It is recommended that BCVA will be conducted twice on the first day and once on the second day. All values will be entered in the eCRF.

For Part II subjects, if the BCVA value at Visit 1 (Screening/Baseline) is ≥ ± 10 letter gain or loss in the study eye compared to the previous XOLARIS study visit (if applicable), then BCVA must be repeated an additional 2 times, resulting in a total of 3 BCVA measures at Visit 1. To facilitate the additional BCVA measures this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF. The highest score will be used to determine subject eligibility.

If the BCVA value at Visit 1 (Screening/Baseline) is < ± 10 letter difference in the study eye compared to the previous XOLARIS study visit, then BCVA will be collected once and will not be repeated.

If subject was not previously in XOLARIS study, BCVA assessments at baseline must be performed in triplicate.

In Part II, it is preferable that the same masked assessor who performs the BCVA assessments during the masked period from Month 3 through Month 12 also perform these assessments at screening.

10.2. Spectral Domain Optical Coherence Tomography (SD-OCT)

SD-OCT will be performed for both eyes at the times indicated in Table 3, Section 16.1. 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 Central Reading Centre (CRC) where the scans will be evaluated; the CRC will enter the data into the Electronic Data Capture (EDC) system. SD-OCT will be used to quantify integrity of the EZ 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).

10.3. Fundus Autofluorescence

To assess changes in the area of viable retinal tissue, fundus autofluorescence will be performed for both eyes at the times indicated in Table 3, Section 16.1. 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 fundus autofluorescence, refer to the Study Operations Manual (which will include procedures from the CRC regarding how measurements are to be taken).

10.4. MAIA Microperimetry

MAIA Microperimetry will be conducted for both eyes at the times indicated in Table 3, Section 16.1. MAIA microperimetry will be conducted by certified technicians to assess changes in retinal sensitivity within the macula. Microperimetry must be performed on both eyes in triplicate and should be conducted over a 2-day period at Visit 1 for all subjects. The final assessment should be used to determine subject eligibility. If at subsequent visits there are obvious technical challenges or the subject is not performing the assessment as expected from previous visits (e.g. distracted, large number of false positive responses, not maintaining fixation, etc), then the assessment may be repeated and the second assessment should be used for that study visit.

All microperimetry images will be sent by the sites to a 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).

In Part II, it is preferable that the same masked assessor who performs the microperimetry assessments during the masked period from Month 3 through Month 12 also perform these assessments at screening.

10.5. Visual Field Testing (Perimetry)

Visual fields will be assessed in both eyes at the times indicated in Table 3, Section 16.1 only at sites where the required perimetry equipment, as specified in the Study Operations Manual, is available. Visual fields will be assessed in triplicate over a 2-day period at Visit 1 for all subjects. Visual field outputs will be sent to a CRC for review. Data will be generated and collated within the CRC and exported to the Sponsor or designee for inclusion in the study database. In Part II, visual fields should be assessed using the Octopus 900 perimeter.

10.6. Contrast Sensitivity

Contrast sensitivity will be measured for both eyes at the times indicated in Table 3, Section 16.1. For contrast sensitivity, assessors will be appropriately qualified for conducting the assessment.

In Part II, it is preferable that the same masked assessor who performs the contrast sensitivity assessments during the masked period from Month 3 through Month 12 also perform these assessments at screening.

10.7. Low Luminance Visual Acuity

Low luminance visual acuity will be measured for both eyes at the times indicated in Table 3, Section 16.1. The test should be performed before BCVA testing and pupil dilation. LLVA is measured by placing a 2.0-log-unit neutral density filter over the front of each eye and having the subject read the normally illuminated ETDRS chart. Initially, letters are read at a distance of 4 meters from the chart. If <20 letters are read at 4 meters, testing at 1 meter should be performed. LLVA is to be reported as number of letters read correctly by the subject.

In Part II, it is preferable that the same masked assessor who performs the LLVA assessments during the masked period from Month 3 through Month 12 also perform these assessments at screening.

[redacted] See Table 3, Section 16.1. [redacted]

11. ASSESSMENT OF SAFETY

All efforts should be made to conduct the safety assessments; 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.

11.1. Dose Limiting Toxicity

See Section 6.1.1 for definitions of DLTs; See Section 11.2.4 for instruction on reporting DLTs.

11.2. Evaluation, Recording, and Reporting Adverse Events

11.2.1. Definitions

11.2.1.1. 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 unfavorable 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 XLRP) or illness that worsens during the study (i.e., increases in frequency or intensity).

11.2.1.2. Serious Adverse Event

An SAE is defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- 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 or for an elective procedure or routinely scheduled treatment for a pre-existing condition, which has not worsened, does not constitute an SAE.

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

Vision Loss to Be Reported as a Serious Adverse Event

Vision Loss NOT TO BE Reported as an SAE:

- Surgery-related BCVA decrease of ≥15 letters on ETDRS chart occurring within1 day of surgery but recovering / resolving at post-operative Day 7.

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 loss is defined as a return to within 10 letters of the baseline BCVA on the ETDRS chart.**

11.2.2. Recording of Adverse Events

AEs/SAEs will be collected from the time the subject or parent / legal guardian (where applicable) provides written informed consent through Visit 11 (or ET Visit or Unscheduled Visits, if applicable).

AEs/SAEs that the investigator becomes aware of, and which are deemed to have a relationship to the study drug, should continue to be reported to NightstaRx following the completion of the study for a period of up to 5 years following surgery, until/unless the subject is enrolled in another NightstaRx study.

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 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.

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 11.2.1.2 for the definition of seriousness). Follow-up information should be provided as necessary (see Section 11.2.3 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:	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:* 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:* 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 *surgical procedure*, *both*, or *unknown*.

11.2.3. 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, stabilized or the subject or parent / legal guardian (where applicable) 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, stabilized or the subject or parent / legal guardian (where applicable) 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.

11.2.4. Reporting of Serious Adverse Events and Dose-Limiting Toxicities

The investigator shall immediately (within 24 hours of learning of the event) report any SAE (and/or DLT) to the Sponsor (or its designee) by completing and emailing the DLT/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 may unmask any SAE reports that are serious, unexpected, and related to the study drug, as required, in accordance with safety reporting guidance and regulations.

The sponsor will report Suspected Unexpected Serious Adverse Reactions (SUSARs) to investigative sites, Institutional Review Boards/Independent Ethics Committees (IRBs/IECs) and regulatory authorities in compliance with current legislation. All cases that are fatal or life-threatening will be reported immediately after the sponsor receives the initial report from the Investigator. All non-fatal or non-life-threatening cases will be reported within a maximum of fifteen days after the initial Investigators 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 DLT/SAE form is provided in the Study Operations Manual, along with the SAE reporting contact information.

11.2.5. Procedures for Unmasking

The Investigator has the ability to unmask an individual subject's treatment assignment to provide emergency treatment. Unmasking is appropriate when knowledge of the subject's dose would affect the medical management of the subject.

If the Investigator determines the unmasking is required, then s/he unmasks the subject through the Electronic Data Capture (EDC) system, files the resulting confirmation in a restricted file until the end of the study, and notifies the Medical Monitor within 24 hours that the subject's treatment assignment has been unmasked. The Investigator will not communicate the treatment assignment to the Medical Monitor or to any sponsor or study team staff; nor should the treatment assignment be communicated to site staff other than those personnel who require that knowledge in order to treat the subject.

The Investigator will ensure that the rationale, date, and time of unmasking is noted in the subject's source documentation, but not the treatment assignment. The medical emergency that necessitated unmasking must also be recorded in the source documentation and the case report form per standard study procedures.

11.2.6. Data Monitoring Committee

For Part I, an independent DMC will be used in this study to safeguard the safety and interests of study subjects and assess the safety and benefit/risk 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 safety aspects of the study, including recommendations for dose escalation (see Section 6.3). The DMC will 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.

In Part II, DMC will be held to review safety and assess benefit/risk during the course of the study.

If one IA is performed, the study will continue as planned in a masked fashion.

11.3. 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 a Pregnancy Notification Form. In addition, if possible, outcome of the pregnancy fathered by the subject should be recorded, including any congenital abnormality or birth defects.

11.4. Full Ophthalmic Examination

A full ophthalmic examination will be conducted for both eyes at the times indicated in Table 3, Section 16.1. The ophthalmic examination will include indirect ophthalmoscopy, slit lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading. The same slit lamp machine and lighting conditions should be used across study visits for any given subject.

Subjects who develop cataracts may undergo cataract surgery if deemed clinically necessary; if surgery is performed, it should be carried out at least 4 weeks before the Visit 9 (Month 12) or Visit 11 (Month 24).

11.5. Fundus Photography

To aid in the objective clinical assessment of progressive retinal changes in the periphery of the retina, fundus photography will be performed for both eyes at the times indicated in Table 3, Section 16.1. Fundus photography will be performed by certified technicians following pupil dilation. 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 (which will include procedures from the CRC regarding how measurements are to be taken).

11.6. Vital Signs

Vital signs (pulse and systolic and diastolic blood pressure) will be taken at the times indicated in Table 3, Section 16.1. Vital signs should be taken after the subject is seated for at least 5 minutes.

11.7. Laboratory Assessments

11.7.1. Laboratory Safety Parameters

Blood samples will be collected at the times indicated in Table 3, Section 16.1 for measurement of hematology and clinical chemistry parameters. 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.

The hematology and clinical chemistry parameters to be evaluated are outlined in Table 2.

Table 2 Laboratory Safety Parameters

Hematology	Clinical Chemistry
Hematocrit	Albumin
Hemoglobin	Alkaline phosphatase
Platelet count	Aspartate transaminase
Red blood cell count	Alanine transaminase
White blood cell count with differential	Bilirubin (total)
	Blood urea nitrogen
	Calcium
	Chloride
	Creatinine
	C-reactive protein
	Gamma glutamyl transferase
	Globulin
	Glucose (non-fasting)
	Lactate dehydrogenase
	Magnesium
	Phosphate
	Potassium
	Protein (total)
	Sodium

11.7.2. Viral Shedding

Blood, tears (both eyes), saliva and urine samples will be collected at the times indicated in Table 3, Section 16.1 and tested by polymerase chain reaction amplification of vector genomes to 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.

11.7.3. Immunogenicity Testing

For the evaluation of immunogenicity, blood will be collected at the times indicated in Table 3, Section 16.1.

Immunoassays are planned to assess antibody and cell-based responses against AAV8-RPGR. Enzyme-linked immunospot (ELISPOT) assays will be used for T-cell mediated immune responses to transgene, and antibody responses will be assayed using enzyme-linked immunosorbent assay-based methods and cell-based methods.

All immunogenicity samples will be sent to central laboratories for analyses. Refer to the Study Operations Manual for details on the shipping and handling of samples.

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

12. STATISTICAL CONSIDERATIONS

For full details of the statistical analyses, please refer to the XIRIUS Statistical Analysis Plan (SAP).

12.1. Sample Size

Due to the nature of the study design of Part I, no formal sample size computation was performed.

In Part II, the primary endpoint is the proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci of the 10-2 grid assessed by MAIA microperimetry at 12 months. A sample size of 10 subjects from a treatment group (either high dose or low dose), and 9 subjects from the untreated control group, will provide approximately 87% power, assuming the treatment group has a 50% response rate and the untreated group has a 5% response rate. The power calculation is based on Fisher Exact-Boschloo test with a Berger-Boos correction of beta = 0.001, at a right-sided significance level of 0.10.

12.2. 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 dropouts and missing observations will depend on their nature and frequency.

Part I is an open-label study. For efficacy endpoints, observations collected within 6 months of the last visit window will be included in the primary analysis. Efficacy endpoints will be summarized on observed data and no imputation will be performed. Safety endpoints will also be analysed on observed data and no imputation will be performed.

Part II is a randomized and double-masked study. For efficacy endpoints, observations collected within 6 months of the last visit window will be included in the primary analysis. Additional imputation approaches will be specified in the SAP. Safety endpoints will be analysed on observed data and no imputation will be performed.

12.3. Analysis Sets

12.3.1. Safety Analysis Set

For Part I, the Safety Analysis Set will consist of all subjects who receive study treatment (vitrectomy/AAV8-RPGR). The Safety Analysis Set will be used for all analyses in Part I.

For Part II, the Safety Analysis Set will consist of all subjects who are randomised under both the 2- and the 3-arm randomisation schedules and receive study treatment when randomized to active treatment, or attend Visit 2 (telephone call) when randomized to control (no treatment). Safety summaries of Part II will use the Safety Analysis Set and subjects will be analysed based on the actual treatment received.

12.3.2. Intent-to-Treat (ITT) Analysis Set

The intent-to-treat (ITT) Analysis Set will consist of all Part II subjects who are randomised, under the 3-arm randomisation schedule. The efficacy summaries of Part II will be generated using the ITT Analysis Set. Subjects will be analysed based on the treatment to which they were randomised.

12.4. Descriptive Statistics

Summary statistics will be presented for both eyes (Study Eyes versus Fellow Eyes). No formal statistical comparison will be performed in Part I. Formal statistical testing will be conducted for the primary endpoint in Part II. Continuous variables and their change from baseline will be summarized using descriptive statistics (number of subjects [n], mean, standard deviation, median, minimum, maximum, first and third quartiles, fifth and ninety-fifth percentiles). The CIs of the mean and the mean change from baseline at each visit may be provided where applicable. For categorical variables, the number and proportion of subjects pertaining to each category will be presented over time. The CIs of the proportion may be provided where applicable.

12.5. Demographics and Baseline Characteristics

Demographics and baseline ocular characteristics will be summarised by Safety Analysis Set in both Part I and Part II

12.6. Efficacy Analyses

Efficacy assessments are ocular in nature and therefore will be tabulated by eye (Study Eye and Fellow Eye). In Part I, efficacy endpoints will be summarised on observed data using descriptive statistics only (described in Section 12.4). In Part II, efficacy endpoints will be summarized on observed data using descriptive statistics. Additional analyses for handling missing data will be provided for the primary endpoint and selected secondary endpoint.

For each subject, data collected within 6 months of the last visit window will be included in the primary analysis, as described in greater detail in the SAP.

At final analysis in Part II, the primary endpoint, which is defined as the proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci at 12 months, will be compared between treatment groups (high dose vs untreated; low dose vs untreated) using the Fisher Exact-Boschloo test with a Berger-Boos correction of beta=0.001 (Berger and Boos 1994). The CIs for the difference in proportions between treatment groups will be presented by Miettinen and Nurminen (Miettinen and Nurminen 1985) method.

For the continuous variables of mean sensitivity, BCVA, and LLVA in Part II, the primary analysis to handle missing data uses a mixed model repeated measures (MMRM) model. The mean change from baseline will be analyzed using fixed effects of treatment group, study visit, study-visit-treatment interaction, and baseline value.

12.6.1. Multiplicity Adjustment

Part I is the dose-escalation to identify the MTD based on the evaluation of benefit/risk without any formal statistical comparisons, and therefore no multiplicity adjustment is needed.

In Part II, a single administrative IA may be included to facilitate the planning of Phase 3 activities. As no early stopping for efficacy is allowed, no multiplicity adjustment is required for this administrative IA. At final analysis, the primary endpoint will be tested using Hochberg's (Hochberg 1988) step-up method with familywise error rate controlled at one-side 0.10. The study will be declared positive if either dose or both doses achieve statistical significance.

12.7. Safety Analyses

Due to the potential systemic effect of study treatment (surgery/study drug) on the contralateral eye, ocular assessments and AEs will be summarised by eye (Study Eye and Fellow Eye) while systemic assessments will be analysed at the subject level. No formal statistical testing will be performed for safety analyses. Safety analyses will be performed on the Safety Analysis Set.

12.7.1. Adverse Events

AEs will be coded using the Medical Dictionary for Regulatory Activities. The version of the dictionary current at the time of the database lock will be used.

AEs will be summarised by system organ class and preferred term. Both the number of eyes/subjects experiencing an AE and the number of events will be summarized. The SAEs, severity, and relationship to study drug/procedure will be also summarized. A by-subject listing of SAEs and DLTs (Part I only) will be provided.

12.7.2. Ocular Safety Evaluations

IOP and changes from baseline in IOP, abnormal slit lamp examination findings and indirect ophthalmoscopy findings, and anterior chamber and vitreous inflammation grading will be summarised by visit and eye.

Lens opacity categories and shifts from baseline will be summarised by visit and eye.

Categories of fundus photography findings (none/mild/moderate/severe) will be summarised by visit and eye.

The number of subjects with a 5, 10 and 15letter decrease from baseline in BCVA will be tabulated by visit and by eye.

12.7.3. Laboratory Assessments and Vital Signs

Laboratory assessments and vital signs will be summarised in a descriptive manner (described in Section 12.4).

12.8. Interim Analysis

[redacted]

In Part II, a single administrative IA may be performed. If this IA is performed, the study will continue as planned in a masked fashion.

13. INFORMED CONSENT, ETHICAL REVIEW AND REGULATORY CONSIDERATIONS

13.1. Informed Consent

Subjects with XLRP who meet all the entry criteria and might be expected to benefit from gene therapy will be invited to take part in the study. Subjects or parent / legal guardian (where applicable) must personally sign and date the latest IEC/ IRB approved version of the informed consent form before any study-specific procedures are performed. Where applicable, a subject assent form will be obtained.

Written and verbal versions of the subject information and informed consent will be presented to the subject or parent / legal guardian (where applicable) 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 or parent /legal guardian (where applicable) 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 or parent / legal guardian (where applicable) dated signature and dated signature of the person who presented and obtained the informed consent. Where applicable, a subject assent form will be obtained. The person who obtained the consent/assent must be suitably qualified and experienced and have been authorized to do so by the investigator. A copy of the signed informed consent will be given to each subject or parent / legal guardian. The original signed form will be retained at the study site and an additional copy will remain in the subject's medical records.

Each subject will have 1 eye treated. Selection of the "study eye" will be made on clinical grounds and will generally be the worse eye affected. This will be discussed in detail and agreed with each subject as part of the informed consent process.

This is an assessor-masked study. All subjects will be informed at the time of randomisation whether they have been randomised to the AAV8-RPGR or Control group. However, subjects in the AAV8-RPGR treatment groups will be masked to the assigned dose level.

In the case of a female partner pregnancy fathered by a subject, a partner pregnancy informed consent form must be signed and dated before collecting information regarding the outcome of the pregnancy (birth or spontaneous abortion), as well as any incidents of congenital abnormality, birth defect or other medical problems.

13.2. Ethical/Regulatory Review

The protocol, informed consent/assent form, subject information sheet and any proposed advertising material will be submitted, in accordance with local requirements, 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.

13.3. Regulatory Considerations

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

The study will be conducted in accordance with the relevant articles of the Declaration of Helsinki, where permissible.

14. ADMINISTRATIVE PROCEDURES

14.1. 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 (SOPs).

The Sponsor and its selected vendors have systems in place for implementing and maintaining quality assurance and quality control systems, with written 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 to independently verify compliance with the protocol.

14.2. 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 individual pass-codes known to each investigator 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 center for centralized, standardised review. Data from these reading centers 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.

14.3. Access to Source Documentation and Subject Privacy

Direct access will be granted to authorized 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 authorized 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 anonymized as soon as it is practical to do so.

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.

14.4. End of the Study

Part I includes 24 months of follow-up for each subject enrolled and treated. Part II includes 12 months of follow-up for each subject enrolled and treated.

The end of the study is defined as the date the last subject completes his final-visit 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.

14.5. Policy for Publication and Presentation of Data

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

15. REFERENCE LIST

Anderson, N. G., M. S. Fineman and G. C. Brown (2006). "Incidence of intraocular pressure spike and other adverse events after vitreoretinal surgery." Ophthalmology **113**(1): 42-47.

Banker, A. S., W. R. Freeman, J. W. Kim, D. Munguia and S. P. Azen (1997). "Vision-threatening complications of surgery for full-thickness macular holes. Vitrectomy for Macular Hole Study Group." Ophthalmology **104**(9): 1442-1452; discussion 1452-1443.

Barry, M. P., G. Dagnelie and I. I. S. G. Argus (2012). "Use of the Argus II retinal prosthesis to improve visual guidance of fine hand movements." Invest Ophthalmol Vis Sci **53**(9): 5095-5101.

Bellingrath, J. S., G. A. Ochakovski, I. P. Seitz, S. Kohl, E. Zrenner, N. Hanig, H. Prokisch, B. H. Weber, S. M. Downes, S. Ramsden, R. E. MacLaren and M. D. Fischer (2017). "High Symmetry of Visual Acuity and Visual Fields in RPGR-Linked Retinitis Pigmentosa." Invest Ophthalmol Vis Sci **58**(11): 4457-4466.

Beltran, W. A., A. V. Cideciyan, S. Iwabe, M. Swider, M. S. Kosyk, K. McDaid, I. Martynyuk, G. S. Ying, J. Shaffer, W. T. Deng, S. L. Boye, A. S. Lewin, W. W. Hauswirth, S. G. Jacobson and G. D. Aguirre (2015). "Successful arrest of photoreceptor and vision loss expands the therapeutic window of retinal gene therapy to later stages of disease." Proc Natl Acad Sci U S A **112**(43): E5844-5853.

Beltran, W. A., A. V. Cideciyan, A. S. Lewin, S. Iwabe, H. Khanna, A. Sumaroka, V. A. Chiodo, D. S. Fajardo, A. J. Román, W. T. Deng, M. Swider, T. S. Alemán, S. L. Boye, S. Genini, A. Swaroop, W. W. Hauswirth, S. G. Jacobson and G. D. Aguirre (2012). "Gene therapy rescues photoreceptor blindness in dogs and paves the way for treating human X-linked retinitis pigmentosa." Proc Natl Acad Sci U S A **109**(6): 2132-2137.

Berger, R. and D. Boos (1994). "P values maximized over a confidence set for the nuisance parameter." J Am Stat Assoc **89**(427): 1012-1016.

Birch, D. G., R. G. Weleber, J. L. Duncan, G. J. Jaffe, W. Tao and G. Ciliary Neurotrophic Factor Retinitis Pigmentosa Study (2013). "Randomized trial of ciliary neurotrophic factor delivered by encapsulated cell intraocular implants for retinitis pigmentosa." Am J Ophthalmol **156**(2): 283-292 e281.

Boye, S. E., S. L. Boye, A. S. Lewin and W. W. Hauswirth (2013). "A comprehensive review of retinal gene therapy." Mol Ther **21**(3): 509-519.

Bryant, J., J. Duker and E. Reichel (2012). "Gene therapy for retinal disease." Review of Ophthalmology.

Chang, S., L. Vaccarella, S. Olatunji, C. Cebulla and J. Christoforidis (2011). "Diagnostic challenges in retinitis pigmentosa: genotypic multiplicity and phenotypic variability." Curr Genomics **12**(4): 267-275.

Cheng, L., S. P. Azen, M. H. El-Bradey, B. M. Scholz, S. Chaidhawangul, M. Toyoguchi and W. R. Freeman (2001). "Duration of vitrectomy and postoperative cataract in the vitrectomy for macular hole study." Am J Ophthalmol **132**(6): 881-887.

Day, T. P., L. C. Byrne, D. V. Schaffer and J. G. Flannery (2014). "Advances in AAV vector development for gene therapy in the retina." Adv Exp Med Biol **801**: 687-693.

Deng, W. T., F. M. Dyka, A. Dinculescu, J. Li, P. Zhu, V. A. Chiodo, S. L. Boye, T. J. Conlon, K. Erger, T. Cossette and W. W. Hauswirth (2015). "Stability and Safety of an AAV Vector for Treating RPGR-ORF15 X-Linked Retinitis Pigmentosa." Hum Gene Ther **26**(9): 593-602.

Edwards, T. L., J. K. Jolly, M. Groppe, A. R. Barnard, C. L. Cottriall, T. Tolmachova, G. C. Black, A. R. Webster, A. J. Lotery, G. E. Holder, K. Xue, S. M. Downes, M. P. Simunovic, M. C. Seabra and R. E. MacLaren (2016). "Visual acuity after retinal gene therapy for choroideremia." N Engl J Med **374**(20): 1996-1998.

EMA (1994). Guideline for Industry. ICH Topic E 4: Dose-Response Information to Support Drug Registration. E. M. Agency.

EMA (2007). Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products, European Medicines Agency (EMA) Committee for Advanced Therapies.

EMA (2016). Concept paper on the revision of the 'Guideline on 4 strategies to identify and mitigate risks for first-in-human 5 clinical trials with investigational medicinal products", European Medicines Agency (EMA) Committee for Advanced Therapies.

EMA (2018). Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products, European Medicines Agency (EMA) Committee for Advanced Therapies.

FDA (2015). Guidance for Industry. Considerations for the design of early-phase clinical trials of cellular and gene therapy products. F. a. D. Administration.

FDA (2018). Human Gene Therapy for Retinal Disorders, Draft Guidance for Industry. F. a. D. Administration.

Ferrari, S., E. Di Iorio, V. Barbaro, D. Ponzin, F. S. Sorrentino and F. Parmeggiani (2011). "Retinitis pigmentosa: genes and disease mechanisms." Curr Genomics **12**(4): 238-249.

Fischer, M. D., M. E. McClements, C. Martinez-Fernandez de la Camara, J. S. Bellingrath, D. Dauletbekov, S. C. Ramsden, D. G. Hickey, A. R. Barnard and R. E. MacLaren (2017). "Codon-Optimized RPGR Improves Stability and Efficacy of AAV8 Gene Therapy in Two Mouse Models of X-Linked Retinitis Pigmentosa." Mol Ther **25**(8): 1854-1865.

Fischer, M. D., B. Wilhelm and S. Michalakis (2016). Successful delivery of rAAV8.CNGA3 in a patient with CNGA3 achromatopsia. Abstract 5207. 2016 Annual Meeting of the Association for Research in Vision and Ophthalmology.

Fokkema, I. F., J. T. den Dunnen and P. E. Taschner (2005). "LOVD: easy creation of a locus-specific sequence variation database using an "LSDB-in-a-box" approach." Hum Mutat **26**(2): 63-68.

Hamel, C. (2006). "Retinitis pigmentosa." Orphanet J Rare Dis **1**: 40.

Hochberg, Y. (1988). "A sharper Bonferroni procedure for multiple tests of significance." Biometrika **75**(4): 800-802.

Hoffman, D. R., D. K. Hughbanks-Wheaton, N. S. Pearson, G. E. Fish, R. Spencer, A. Takacs, M. Klein, K. G. Locke and D. G. Birch (2014). "Four-year placebo-controlled trial of docosahexaenoic acid in X-linked retinitis pigmentosa (DHAX trial): a randomized clinical trial." JAMA Ophthalmol **132**(7): 866-873.

Hong, D. H. and T. Li (2002). "Complex expression pattern of RPGR reveals a role for purine-rich exonic splicing enhancers." Invest Ophthalmol Vis Sci **43**(11): 3373-3382.

Hong, D. H., B. Pawlyk, M. Sokolov, K. J. Strissel, J. Yang, B. Tulloch, A. F. Wright, V. Y. Arshavsky and T. Li (2003). "RPGR isoforms in photoreceptor connecting cilia and the transitional zone of motile cilia." Invest Ophthalmol Vis Sci **44**(6): 2413-2421.

Hughbanks-Wheaton, D. K., D. G. Birch, G. E. Fish, R. Spencer, N. S. Pearson, A. Takacs and D. R. Hoffman (2014). "Safety assessment of docosahexaenoic acid in X-linked retinitis pigmentosa: the 4-year DHAX trial." Invest Ophthalmol Vis Sci **55**(8): 4958-4966.

Kirschner, R., T. Rosenberg, R. Schultz-Heienbrok, S. Lenzner, S. Feil, R. Roepman, F. P. Cremers, H. H. Ropers and W. Berger (1999). "RPGR transcription studies in mouse and human tissues reveal a retina-specific isoform that is disrupted in a patient with X-linked retinitis pigmentosa." Hum Mol Genet **8**(8): 1571-1578.

MacLaren, R. E., M. Groppe, A. R. Barnard, C. L. Cottriall, T. Tolmachova, L. Seymour, K. R. Clark, M. J. During, F. P. Cremers, G. C. Black, A. J. Lotery, S. M. Downes, A. R. Webster and M. C. Seabra (2014). "Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial." Lancet **383**(9923): 1129-1137.

MacLaren, R. E., R. A. Pearson, A. MacNeil, R. H. Douglas, T. E. Salt, M. Akimoto, A. Swaroop, J. C. Sowden and R. R. Ali (2006). "Retinal repair by transplantation of photoreceptor precursors." Nature **444**(7116): 203-207.

Maguire, A. M., F. Simonelli, E. A. Pierce, E. N. Pugh, Jr., F. Mingozzi, J. Bennicelli, S. Banfi, K. A. Marshall, F. Testa, E. M. Surace, S. Rossi, A. Lyubarsky, V. R. Arruda, B. Konkle, E. Stone, J. Sun, J. Jacobs, L. Dell'Osso, R. Hertle, J. X. Ma, T. M. Redmond, X. Zhu, B. Hauck, O. Zelenaia, K. S. Shindler, M. G. Maguire, J. F. Wright, N. J. Volpe, J. W. McDonnell, A. Auricchio, K. A. High and J. Bennett (2008). "Safety and efficacy of gene transfer for Leber's congenital amaurosis." N Engl J Med **358**(21): 2240-2248.

Mavlyutov, T. A., H. Zhao and P. A. Ferreira (2002). "Species-specific subcellular localization of RPGR and RPGRIP isoforms: implications for the phenotypic variability of congenital retinopathies among species." Hum Mol Genet **11**(16): 1899-1907.

Megaw, R. D., D. C. Soares and A. F. Wright (2015). "RPGR: Its role in photoreceptor physiology, human disease, and future therapies." Exp Eye Res **138**: 32-41.

Miettinen, O. and M. Nurminen (1985). "Comparative analysis of two rates." Stat Med **4**(2): 213-226.

Nussenblatt, R. B., A. G. Palestine, C. C. Chan and F. Roberge (1985). "Standardization of vitreal inflammatory activity in intermediate and posterior uveitis." Ophthalmology **92**(4): 467-471.

Park, S. S., D. M. Marcus, J. S. Duker, R. D. Pesavento, T. M. Topping, A. R. Frederick, Jr. and D. J. D'Amico (1995). "Posterior segment complications after vitrectomy for macular hole." Ophthalmology **102**(5): 775-781.

Pawlyk, B. S., O. V. Bulgakov, X. Sun, M. Adamian, X. Shu, A. J. Smith, E. L. Berson, R. R. Ali, S. Khani, A. F. Wright, M. A. Sandberg and T. Li (2016). "Photoreceptor rescue by an abbreviated human RPGR gene in a murine model of X-linked retinitis pigmentosa." Gene Ther **23**(2): 196-204.

Pelletier, V., M. Jambou, N. Delphin, E. Zinovieva, M. Stum, N. Gigarel, H. Dollfus, C. Hamel, A. Toutain, J. L. Dufier, O. Roche, A. Munnich, J. P. Bonnefont, J. Kaplan and J. M. Rozet (2007). "Comprehensive survey of mutations in RP2 and RPGR in patients affected with distinct retinal dystrophies: genotype-phenotype correlations and impact on genetic counseling." Hum Mutat **28**(1): 81-91.

Petrs-Silva, H. and R. Linden (2014). "Advances in gene therapy technologies to treat retinitis pigmentosa." Clin Ophthalmol **8**: 127-136.

Recchia, F. M., I. U. Scott, G. C. Brown, M. M. Brown, A. C. Ho and M. S. Ip (2010). "Small-gauge pars plana vitrectomy: a report by the American Academy of Ophthalmology." Ophthalmology **117**(9): 1851-1857.

Schmid, F., E. Glaus, F. P. Cremers, B. Kloeckener-Gruissem, W. Berger and J. Neidhardt (2010). "Mutation- and tissue-specific alterations of RPGR transcripts." Invest Ophthalmol Vis Sci **51**(3): 1628-1635.

Shu, X., E. McDowall, A. F. Brown and A. F. Wright (2008). "The human retinitis pigmentosa GTPase regulator gene variant database." Hum Mutat **29**(5): 605-608.

Stein, J. D., D. N. Zacks, D. Grossman, H. Grabe, M. W. Johnson and F. A. Sloan (2009). "Adverse events after pars plana vitrectomy among medicare beneficiaries." Arch Ophthalmol **127**(12): 1656-1663.

Storer, B. E. (1989). "Design and analysis of phase I clinical trials." Biometrics **45**(3): 925-937.

Talcott, K. E., K. Ratnam, S. M. Sundquist, A. S. Lucero, B. J. Lujan, W. Tao, T. C. Porco, A. Roorda and J. L. Duncan (2011). "Longitudinal study of cone photoreceptors during retinal degeneration and in response to ciliary neurotrophic factor treatment." Invest Ophthalmol Vis Sci **52**(5): 2219-2226.

Tee, J. J., A. J. Smith, A. J. Hardcastle and M. Michaelides (2016). "RPGR-associated retinopathy: clinical features, molecular genetics, animal models and therapeutic options." Br J Ophthalmol **100**(8): 1022-1027.

Thompson, J. T., R. N. Sjaarda, B. M. Glaser and R. P. Murphy (1996). "Increased intraocular pressure after macular hole surgery." Am J Ophthalmol **121**(6): 615-622.

Vandenberghe, L. H., P. Bell, A. M. Maguire, C. N. Cearley, R. Xiao, R. Calcedo, L. Wang, M. J. Castle, A. C. Maguire, R. Grant, J. H. Wolfe, J. M. Wilson and J. Bennett (2011). "Dosage thresholds for AAV2 and AAV8 photoreceptor gene therapy in monkey." Sci Transl Med **3**(88): 88ra54.

Vervoort, R., A. Lennon, A. C. Bird, B. Tulloch, R. Axton, M. G. Miano, A. Meindl, T. Meitinger, A. Ciccodicola and A. F. Wright (2000). "Mutational hot spot within a new RPGR exon in X-linked retinitis pigmentosa." Nat Genet **25**(4): 462-466.

Webb, T. R., D. A. Parfitt, J. C. Gardner, A. Martinez, D. Bevilacqua, A. E. Davidson, I. Zito, D. L. Thiselton, J. H. Ressa, M. Apergi, N. Schwarz, N. Kanuga, M. Michaelides, M. E. Cheetham, M. B. Gorin and A. J. Hardcastle (2012). "Deep intronic mutation in OFD1, identified by targeted genomic next-generation sequencing, causes a severe form of X-linked retinitis pigmentosa (RP23)." Hum Mol Genet **21**(16): 3647-3654.

Wen, R., W. Tao, Y. Li and P. A. Sieving (2012). "CNTF and retina." Prog Retin Eye Res **31**(2): 136-151.

West, E. L., R. A. Pearson, Y. Duran, A. Gonzalez-Cordero, R. E. MacLaren, A. J. Smith, J. C. Sowden and R. R. Ali (2012). "Manipulation of the recipient retinal environment by ectopic expression of neurotrophic growth factors can improve transplanted photoreceptor integration and survival." Cell Transplant **21**(5): 871-887.

Wu, Z., S. Hiriyanna, H. Qian, S. Mookherjee, M. M. Campos, C. Gao, R. Fariss, P. A. Sieving, T. Li, P. Colosi and A. Swaroop (2015). "A long-term efficacy study of gene replacement therapy for RPGR-associated retinal degeneration." Hum Mol Genet **24**(14): 3956-3970.

Yan, D., P. K. Swain, D. Breuer, R. M. Tucker, W. Wu, R. Fujita, A. Rehemtulla, D. Burke and A. Swaroop (1998). "Biochemical characterization and subcellular localization of the mouse retinitis pigmentosa GTPase regulator (mRpgr)." J Biol Chem **273**(31): 19656-19663.

Zhou, W. T., Y. Q. Ni, Z. B. Jin, M. Zhang, J. H. Wu, Y. Zhu, G. Z. Xu and D. K. Gan (2012). "Electrical stimulation ameliorates light-induced photoreceptor degeneration in vitro via suppressing the proinflammatory effect of microglia and enhancing the neurotrophic potential of Muller cells." Exp Neurol **238**(2): 192-208.

Zrenner, E., K. U. Bartz-Schmidt, H. Benav, D. Besch, A. Bruckmann, V. P. Gabel, F. Gekeler, U. Greppmaier, A. Harscher, S. Kibbel, J. Koch, A. Kusnyerik, T. Peters, K. Stingl, H. Sachs, A. Stett, P. Szurman, B. Wilhelm and R. Wilke (2011). "Subretinal electronic chips allow blind patients to read letters and combine them to words." Proc Biol Sci **278**(1711): 1489-1497.

16. APPENDICES

16.1. Schedule of Study Procedures

Table 3 presents a schedule of study procedures.

Table 3 Schedule of Study Procedures

Study Visit	Screening/ Baseline[a]	Day 0	Day 1	Day 7	Month 1	Month 2	Month 3	Month 6	Month 9	Month 12[b] Year 1	Month 18[c]	Month 24 Year 2[c,d]	ET Visit[e]	Uns. Visit[f]
Visit Window				**(±3d)**	**(±7d)**	**(±7d)**	**(±7d)**	**(±14d)**	**(±14d)**	**(±14d)**	**(±14d)**	**(±14d)**		
Visit Number	**Visit 1**	**Visit 2[g]**	**Visit 3[g]**	**Visit 4[g]**	**Visit 5**	**Visit 5.9**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**	**Visit 10**	**Visit 11**		
Assessments/Procedures (All Subjects/ Both Eyes, Unless Otherwise Specified)														
Informed consent/assent	X													
Demography	X													
Medical history, incl ocular history	X													
Blood pressure	X	X	X	X										
Pulse	X	X	X	X										
Safety blood samples[h]	X			X	X	X	X			X		X	X	
RPGR mutation screen[i]	X													
Full ophthalmic examination[j]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Surgical procedure/dosing[k]		X												
Dispensation of oral steroids[l]	X													
ETDRS BCVA[m]	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	X	X
LLVA	X				X		X	X	X	X	X	X	X	
Fundus autofluorescence	X				X	X	X	X		X	X	X	X	X

Study Visit	Screening/ Baseline[a]	Day 0	Day 1	Day 7	Month 1	Month 2	Month 3	Month 6	Month 9	Month 12[b] Year 1	Month 18[c]	Month 24 Year 2[c,d]	ET Visit[e]	Uns. Visit[f]
Visit Window				(±3d)	(±7d)	(±7d)	(±7d)	(±14d)	(±14d)	(±14d)	(±14d)	(±14d)		
Visit Number	Visit 1	Visit 2[g]	Visit 3[g]	Visit 4[g]	Visit 5	Visit 5.9	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11		
MAIA Microperimetry[n]	X				X	X	X	X	X	X	X	X	X	X
Fundus photography	X									X	X	X	X	
Visual fields (Octopus 900 in Part II)[o]	X						X	X		X		X	X	
[redacted]														
Contrast sensitivity	X						X	X		X		X	X	
Viral shedding[q]	X		X	X	X	X								
Immunogenicity sampling[r]	X		X	X	X	X	X	X		X			X	
AE, SAE monitoring[s]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication, treatments, and procedures review	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Corticosteroid compliance review[t]		X	X	X	X	X								
Randomization	X													
[redacted]														

Abbreviations: AE=adverse event; BCVA=best-corrected visual acuity; ET=early termination; ETDRS=Early Treatment of Diabetic Retinopathy Study; IOP=intraocular pressure; LOCS III =Lens Opacities Classification System III; LLVA= Low luminance visual acuity; SAE=serious adverse event; SD-OCT=spectral domain optical coherence tomography; [redacted]; [redacted]

All procedures will be performed for both eyes, unless otherwise specified.

a. The Screening/Baseline Visit must be performed within 12 weeks of Visit 2
b. Although the visit will still be considered out-of-window, if a subject in Part II is unable to attend the 12-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.
c. Visits 10 and 11 are for Part I subjects only

d. Although the visit will still be considered out-of-window, if a subject in Part I is unable to attend the 24-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.
e. An early termination (ET) visit is to be performed if a subject discontinues at any time. [redacted]
f. If clinically indicated, subjects may need to return to the site for an unscheduled visit. At a minimum, the following assessments will be performed: full ophthalmic examination, BCVA, SD-OCT, microperimetry (if clinically feasible), fundus autofluorescence, AE/SAE monitoring, and concomitant medication, treatments, and procedures review
g. Visits 2, 3, and 4 will be a telephone call only for subjects randomised to the control, untreated group. AE/SAE monitoring and concomitant medication, treatments, and procedures review will be assessed during the call.
h. Includes hematology and clinical chemistry
i. To be conducted only if unavailable at Visit 1
j. Includes indirect ophthalmoscopy, slit lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading
k. Study eye only
l. Part I subjects will take 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). Part II subjects in treated groups will be given a 9-week course of oral prednisone/prednisolone and instructed to start taking the drug 3 days before Visit 2. See Section 8.8.
m. Part I subjects perform BCVA assessments in triplicate at baseline, Month 12, Month 24, and at the Early Termination visit, when applicable. For Part II subjects only: if the BCVA value at Visit 1 (Screening/Baseline) is ≥ ± 10 letter gain or loss in the study eye compared to the previous XOLARIS study visit (if applicable), then BCVA must be repeated an additional 2 times, resulting in a total of 3 BCVA measures at Visit 1.
In Part II subjects, if the BCVA value at Visit 1 (Screening/Baseline) is < ± 10 letter difference in the study eye compared to the previous XOLARIS study visit, then BCVA will be collected once and will not be repeated. If subject was not previously in XOLARIS study, BCVA assessments at baseline must be triplicate.
For all subjects who require triplicate BCVA testing, to facilitate the additional BCVA measures, this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF. At screening, the highest score will be used to define subject eligibility.
n. Subjects perform triplicate microperimetry only at baseline. The final microperimetry assessment will be used to determine eligibility. Microperimetry should be conducted at unscheduled visits, if clinically feasible. Microperimetry outputs will be sent to a CRC for review. Data will be generated and collated within the CRC and exported to the Sponsor or designee for inclusion in the study database.
o. Triplicate at baseline. Octopus 900 perimeter is specified for visual field assessments only in Part II. Visual field outputs will be sent to a CRC for review. Data will be generated and collated within the CRC and exported to the Sponsor or designee for inclusion in the study database.
[redacted]
q. Blood, tears (both eyes), saliva, and urine samples will be collected for the viral shedding assay.
r. Immunogenicity sampling at the ET Visit is to be conducted only if visit occurs prior to Month 12 visit.
s. AEs/SAEs will be collected from the time the subject provides written informed consent/assent through to the last study visit (or ET Visit if applicable).
t. Corticosteroid review is applicable only for treated subjects.
u. Part II only.
[redacted]

Biogen MA Inc.
225 Binney Street
Cambridge, MA 02142
United States

Biogen Idec Research Limited
Innovation House
70 Norden Road
Maidenhead, Berkshire
SL6 4AY
United Kingdom

AMENDMENT SUMMARY

Biogen Protocol 274RP101 (NSR-RPGR-01)

A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

XIRIUS Study

For Global Protocol Amendment: Version 11

Date: 1 Oct 2020

EUDRA CT Number: 2016-003852-60

Version 11 of the protocol has been prepared for this amendment, which supersedes Version 10, 10 October 2019.

PRIMARY REASON FOR AMENDMENT

Two primary reasons for this amendment to Protocol 274RP101 (NSR-RPGR-01) are 1) to reduce the sample size and 2) remove the requirement for an interim analysis (IA).

Regarding the first primary reason, the sections most affected by these changes are Section 13.1 (Sample Size), and secondarily Sections 7.1 (Overall Study Design), Section 7.1.4 (Part II Dose Expansion), Section 7.2, (Number of Subjects). This change in sample size required additional modifications to the planned Statistical Analyses. The sections most affected by the statistical analysis changes are Section 13.2 (Procedure for Accounting for Missing Data), Section 13.3.1 (Safety Analysis Set), Section 13.3.2 (Intent to Treat Analysis Set), Section 13.4 (Descriptive Statistics), Section 13.5 (Demographics and Baseline Characteristics), Section 13.6 (Efficacy Analysis), Section 13.6.1 (Multiplicity Adjustment), Section 13.7.1 (Adverse Events), and Section 13.7.2 (Ocular Safety).

Regarding the second primary reason, the sections most affected by these changes are Section 13.8 (Interim Analysis), and secondarily, Sections 7.1.4 (Part II, Dose-Expansion), Section 12.2.6 (Data Monitoring Committee).

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

1. PRIMARY REASON ONE

Section 13.1, Sample Size

~~In Part II, a sample size of 45 subjects, 15 in each of 3 groups (high-dose, low-dose and untreated), ensures 80% power at 0.05 significance level assuming that the treated arm has 50% probability of achieving ≥7 dB improvement at ≥5 loci at Month 3 vs. 5% in the untreated control arm.~~ **In Part II, the primary endpoint is the proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci of the 10-2 grid assessed by MAIA microperimetry at 12 months. A sample size of 10 subjects from a treatment group (either high dose or low dose), and 9 subjects from the untreated control group, will provide approximately 87% power, assuming the treatment group has a 50% response rate and the untreated group has a 5% response rate. The power calculation is based on Fisher Exact-Boschloo test with a Berger-Boos correction of beta = 0.001, at a right-sided significance level of 0.10.**

Rationale for reduction in sample size:

Study drug expired end of September 2019, and it was not possible to extend the shelf-life, nor was there the possibility of using a new drug supply without causing a significant delay to the completion of this study.

The team performed an assessment and determined to close enrollment as of the last subject enrolled on 30 September 2019. It was decided that 29 subjects will provide sufficient data to inform the next pivotal study.

This change also affects text in Section 7.1 (Overall Study Design), 7.1.4 (Part II, Dose Expansion), and 7.2 (Number of Subjects). This sample size change also triggered broad changes to the planned statistical analyses, affecting Section 13.2 (Procedures for Accounting for Missing Data), Section 13.3.1 (Safety Analysis Set), Section 13.3.2 (Intent to Treat Analysis Set), Section 13.4 (Descriptive Statistics), Section 13.5 (Demographics and Baseline Characteristics), and Section 13.6 (Efficacy Analysis).

Section 7.1, Overall Study Design

Now reads:

Part II will expand 2 doses, allowing for a broader assessment of the safety and efficacy of AAV8-RPGR with a larger sample size, including ~~45~~ **approximately 30** subjects randomized 1:1:1 to a high-dose, a low-dose, or an untreated arm.

Section 7.1.4, Part II, Dose expansion

Now reads:

~~Up to 45~~ **From version 9.0 thereafter, approximately 30** additional subjects will be randomized in a 1:1:1 allocation ratio to a high-dose group (2.5 × 10^11 gp), a low-dose group (5 × 10^10 gp), and an untreated group.

Section 7.2, Number of Subjects

Now reads:

Overall, the study is expected to enroll approximately ~~63~~ 48 subjects: 18 in Part I and ~~up to~~ **approximately** ~~45~~ **30** in Part II **under 1:1:1 randomization scheme**. See Section 13.1 for rationale.

Section 13.2 Procedures for Accounting for Missing Data

Now reads:

~~Safety and efficacy data will be analysed on observed data only. Missing data will not be imputed.~~

Part I is an open-label study. For efficacy endpoints, observations collected within 6 months of the last visit window will be included in the primary analysis. ~~and.~~ **Efficacy endpoints will be summarized on observed data and no imputation will be performed. Safety endpoints will be analysed on observed data and no imputation will be performed.**

Part II is a randomized and double-masked study. For efficacy endpoints, observations collected within 6 months of the last visit window will be included in the primary analysis. Additional imputation approaches will be specified in the SAP. Safety endpoints will be analysed on observed data and no imputation will be performed.

Section 13.3.1, Safety Analysis Set

Now reads:

The Safety Analysis Set will be ~~the primary population for demographics, baseline characteristics and safety analyses~~ used for **all analyses in Part I.**

For Part II, the Safety Analysis Set will consist of all subjects who are randomised , under both the 2- and the 3-arm randomisation schedules and **receive study treatment when randomized to active treatment, or attend Visit 2 (telephone call) when randomized to control (no treatment). Safety summaries of Part II will use the Safety Analysis Set and subjects will be analysed based on the actual treatment received.** ~~The Safety Analysis Set will be the primary population for demographics, baseline characteristics and safety analyses. Subjects will be analysed based on the actual treatment received.~~

Section 13.3.2 Intent to Treat Analysis Set

Now reads:

The intent-to-treat (ITT) Analysis Set will consist of all Part II subjects who are randomised, under the 3-arm randomisation schedules. ~~The ITT Analysis Set will be used for the efficacy analyses.~~ **The efficacy summaries of Part II will be generated using the ITT Analysis Set.** Subjects will be analysed based on the treatment to which they were randomised.

~~The modified intent-to-treat (mITT) Analysis Set will include all Part II subjects who are randomised under the 3-arm randomisation schedule and who receive study treatment (or the phone call for those in the untreated control group) and who have baseline microperimetry data and at least 1 post-baseline microperimetry data in the study eye. The mITT Analysis Set will be used for supportive and sensitivity analysis for Part II primary and secondary efficacy endpoints. Subjects will be analysed based on the treatment to which they were randomised.~~

Section 13.4 Descriptive Statistics

Now reads:

Summary statistics will be presented for both eyes (Study Eyes versus Fellow Eyes). No formal statistical comparison will be performed in Part I, ~~and statistical tests will be conducted for the efficacy endpoints in Part II. For categorical/binary data, the number and proportion of subjects pertaining to each category will be presented over time with its 95% confidence interval (CI). Continuous data will be summarised over time using mean, and its 95% CI, standard deviation, median, minimum and maximum. 95% CIs will be 2-sided. Summaries will be generated by dose and overall, in Part I and, by group (high-dose, low-dose, untreated) in Part II.~~ **Formal statistical testing will be conducted for the primary endpoint in Part II. Continuous variables and their change from baseline will be summarized using descriptive statistics (number of**

subjects [n], mean, standard deviation, median, minimum, maximum, first and third quartiles, fifth and ninety-fifth percentiles). The confidence interval (CI) is of the mean and the mean change from baseline at each visit may be provided where applicable. For categorical variables, the number and proportion of subjects pertaining to each category will be presented over time. The CIs of the proportion may be provided where applicable. ~~will be computed using the Clopper-Pearson method.~~

Section 13.5, Demographics and Baseline Characteristics

Now reads:

Demographics and baseline ocular characteristics will be summarised **by using** for the Safety Analysis Set **in both Part I and Part II.** ~~and the full analysis set for Part I and Part II separately.~~

Section 13.6 Efficacy Analysis

Now reads:

Efficacy assessments are ocular in nature and therefore will be tabulated by eye (Study Eye and Fellow Eye). **In Part I, efficacy** ~~data e~~**ndpoints** will be summarised **on observed data** using descriptive statistics only (described in Section 13.4). **In Part II, efficacy endpoints will be summarized on observed data using descriptive statistics. Additional analyses for handling missing data will be provided for the primary endpoint and selected secondary endpoint.** ~~This will be done for each cohort in Part I, and each arm~~ **group** ~~in Part II.~~

For each subject, data collected within 6 months of the last visit window will be included in the primary analysis, as described in greater detail in the SAP.

~~Improvement in retinal sensitivity and change from baseline in retinal sensitivity will be tabulated by visit and by eye in each Part, and be compared between the randomized groups in Part II by visit for the study eye.~~

At final analysis in Part II, the primary endpoint, which is defined as the proportion of study eyes with ~~with improved retinal sensitivity, for both the center grid (i.e., the central 16 loci) and the entire grid (i.e., all 68 loci),~~ **≥7 dB improvement from baseline at ≥5 of the 16 central loci at 12 months,** will be compared between ~~study arms~~ treatment groups (high dose vs untreated; low dose vs untreated) using the Fisher Exact-Boschloo test with a Berger-Boos correction of beta=0.001 (Berger 1994). ~~In addition, the difference in proportions between study arms will be presented with its corresponding 95% CI calculated~~**The CIs for the** difference in proportions between **treatment groups** will be presented **by** Miettinen and Nurminen (Miettinen 1985) **method**.

For the continuous variables of mean sensitivity, BCVA, and LLVA in Part II, the primary analysis to handle missing data uses a mixed model repeated measures (MMRM) model.

The mean change from baseline will be analyzed using fixed effects of treatment group, study visit, study-visit-treatment interaction, and baseline value.

~~Change from baseline in mean sensitivity, in both the center grid and the entire grid, will be compared between study arms using an ANCOVA model including baseline value and study arm (high dose, low-dose, and untreated) as covariates. The difference in means between study arms, and its 95% CI, will be derived from the same ANCOVA model.~~

Section 13.7.1, Adverse Events

Now reads:

The SAEs, **severity, and relationship to study drug/procedure will be also summarized. A by-subject listing of SAEs and DLTs (Part I only) will be provided.** ~~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.~~

~~For Part I only, A a by-subject listing of DLTs will be prepared.~~

Section 13.7.2 Ocular Safety Evaluation

Now reads:

The number of subjects with a **5,** 10- and 15-letter decrease from baseline in BCVA will be tabulated by visit and by eye.

2. PRIMARY REASON TWO

Section 13.8 Interim Analysis

Now reads:

[redacted]

In Part II, ~~after all subjects complete the 3-month visit,~~ **a single** administrative IA ~~will~~ **may be** performed. ~~by an independent DMC. The DMC will review the accrued safety and efficacy data. A small group of unmasked Sponsor personnel, who will not be involved in the conduct of the study moving forward, will have access to unmasked data for this administrative IA to enable regulatory interactions and planning for phase 3 initiation. The details of the roles and responsibilities of the Part II DMC are provided in the DMC charter, and the full details of masking are provided in the study Masking Plan.~~

If this IA is performed, ~~The~~ the study will continue as planned **in a masked fashion.** ~~A final analysis will be conducted at the end of the study, i.e., after all subjects complete the study. The end of the study is defined as the date the last subject completes his final-visit 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.~~

Rationale: The interim analysis was determined to no longer be necessary.

This change also affects Section 7.1.4, Part II: Dose Expansion

Now reads:

~~After all Part II subjects complete the 3-month visit~~ **During Part II**, **a single** administrative IA ~~will~~ **may** be conducted. **If this IA is performed,** the study will continue as planned **in a masked fashion.**

Section 9.6, Study Masking

Now reads:

~~After all~~**During** Part II, ~~subjects complete the 3-month visit,~~ **one** administrative IA ~~will~~ **may be** performed. ~~by an independent DMC, who will review the accrued safety and efficacy data. A small group of unmasked Sponsor personnel, who will not be involved in the conduct of the study moving forward, will have access to unmasked data for this administrative IA to enable regulatory interactions and planning for phase 3 initiation.~~ **If this IA is performed, the study will continue as planned in a masked fashion.**

~~The operations team, the investigators, medical monitor, and other personnel who will continue to be involved in the daily operation of the study, as well as the subjects, will continue to be masked to the subjects' dose level assignment and outcomes of the IA until study completion to~~

~~minimise any operational bias. Furthermore, the clinical assessors will continue to be masked to the treatment assignment. The full details of masking are provided in the study Masking Plan.~~

Section 12.2.6, Data Monitoring Committee

Now reads:

In Part II, DMC will be held to review safety and assess benefit/risk during the course of the study.

~~After all~~During Part II subjects complete the 3-month visit, ~~an administrative IA will~~ may be ~~performed.~~ **If one IA is performed, the study will continue as planned in a masked fashion.** ~~by an independent DMC, who will review the accrued safety and efficacy data. A small group of unmasked Sponsor personnel, who will not be involved in the conduct of the study moving forward, will have access to unmasked data for this administrative IA to enable regulatory interactions and planning for phase 3 initiation. Following the administrative IA, the DMC will continue to meet, as described in the DMC Charter.~~

Section 13.6.1, Multiplicity Adjustment

Now reads:

In Part II, **a single** administrative IA is ~~**may be** included to facilitate the planning of Phase 3 activities.

~~The final analysis will be conducted at 0.05 two-sided level for the primary endpoint.~~

At final analysis, the primary endpoint will be tested using Hochberg's (Hochberg 1988) step-up method with familywise error rate controlled at < 0.10 (one-sided). The study will be declared positive if either dose or both doses achieve statistical significance.

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Synopsis

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

Section 5.4

Change: Interim data were presented in the original protocol. This is now removed.

Now reads:

~~Interim (4-week) r~~**R**esults from the sponsor's toxicity study also indicate that administration of the same vector (AAV8 RPGR) is well tolerated in male C57BL/6J mice at dose levels of 1×10^9 and 3.54×10^9 gp/eye; ~~interim (35-day)~~ results from the sponsor's biodistribution study also support a lack of toxicity.

Rationale: The nonclinical toxicity studies are now complete.

Section 6.2.1.2, Secondary and Exploratory Endpoints, Part I

Change: Removed Month 9 from the timepoints at which this endpoint will be assessed.

Now reads:

• Change from baseline in fundus autofluorescence at 1, 2, 3, 6, ~~9~~ and 12 months

Rationale: One time point (month 9) was removed from the endpoint analysis regarding autofluorescence as it was added in error. This measure is not assessed at Month 9.

Section 6.2.2.3 Secondary Endpoints

Change: The secondary endpoints have been updated to separate the central 16-loci microperimetry mean change from baseline from the 68-loci microperimetry mean change from baseline. Previously, these 2 separate endpoints were not defined. Also, visual field-related endpoints at 1, 2 and 9 months were listed in error. Visual field is not tested at these visits.

Now reads:

• **Change from baseline in retinal sensitivity at the central 16 loci assessed by MAIA microperimetry at 1, 2, 3, 6, 9, and 12 months**

• Change from baseline in **retinal sensitivity at 68 loci assessed by MAIA** microperimetry at 1, 2, 3, 6, 9, and 12 months

• Change from baseline in visual field assessed by Octopus 900 perimeter at ~~1, 2,~~ 3, 6, ~~9,~~ and 12 months

Rationale: Microperimetry is the most important efficacy endpoint in the study. More granularity was needed as multiple analyses will be conducted on this measure.

Section 6.2.2.4 Exploratory Endpoints

Change: [redacted]

Now reads:

• [redacted]

Rationale: [redacted]

Section 7.1 Overall Study Design

Change: additional time points were added for when microperimetry and safety and secondary efficacy assessments will be evaluated.

Now reads:

Baseline in microperimetry, evaluated at ~~3~~ **12** months, and safety and secondary efficacy evaluated at 1-, **2-, 3-**, 6-, 9-, and 12-months post-treatment.

Rationale: These time points were omitted in error.

Section 7.1 Overall Study Design

Change: Text added regarding missed visits.

Now reads:

Although the visit will still be considered out-of-window, if Part I subjects are unable to attend their final 24-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. Delays will be identified as protocol deviations (PDs) on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

A Part II subject is considered to have completed the study if he completes the Month 12 assessments. **Although the visit will still be considered out-of-window, if Part II subjects are unable to attend their final 12-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. Delays will be identified as protocol deviations (PDs) on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.**

Rationale: Instructions are provided in the case of missed final visits. If a subject misses the 12- or 24-month visit (for Parts I and II), the site is instructed to bring the subject back to complete the 12- or 24-month visit assessments when it is possible to do so, even if this visit will be out-of-window.

This change also affects Section 7.1.2, Part I, Dose Escalation Study, Section 7.1.4, Part II: Dose-Expansion, Section 10.10, Visit 9, Section 10.12, Visit 11.

Section 7.1.2, Part I, Dose Escalation Study

Although the visit will still be considered out-of-window, if Part I subjects are unable to attend their final 24-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. This will be considered their 24-month visit. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

Section 7.1.4, Part II Dose Expansion

Although the visit will still be considered out-of-window, if Part II subjects are unable to attend their final 12-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. This will be considered their 12-month visit. Delays will be identified as PDs on a case-by-case basis by

the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

Section 10.10, Visit 9

Although the visit will still be considered out-of-window, if Part II subjects are unable to attend their final 12-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. This will be considered their 12-month visit. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

Section 10.12, Visit 11

Although the visit will still be considered out-of-window, if Part I subjects are unable to attend their final 24-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. This will be considered their 24-month visit. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

Section 17.1, Schedule of Study Procedures, Footnotes b and d

Now read:

b. Although the visit will still be considered out-of-window, if a subject in Part II is unable to attend the 12-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

d. Although the visit will still be considered out-of-window, if a subject in Part I is unable to attend the 24-month visit during the protocol-defined window, they should return to the site to complete this visit as soon as it is possible for them to do so. Delays will be identified as PDs on a case-by-case basis by the Sponsor based on information provided by the site; this will include but is not limited to scenarios such as site closure, travel bans, medical travel prohibitions, patient illness, etc.

Section 7.3 Discussion of Study Design and Dose Selection

Change: The NOAEL was changed from greater than to equal to 3.54 × 10^9.

Now reads:

The NOAEL was determined to be ~~greater than~~ 3.54 × 10^9 gp/eye in mice, providing a 700-fold safety margin compared to the starting dose.

Rationale: The NOAEL was incorrectly stated.

Section 9.6, Study Masking

Change: Removed masked assessors

In Part II, ophthalmic assessments that will be used as efficacy endpoints (BCVA, LLVA, microperimetry, contrast sensitivity [redacted]) will be conducted by appropriately qualified ~~masked~~ assessors

Rationale: Efficacy assessments are not masked throughout the study, only at certain timepoints. The table linked to this text specifies which timepoints and is therefore correct. However, it has been removed from this statement for accuracy.

Section 15.4 Time and Schedule of the Study

Now reads:

Section 15.4 ~~Time and Schedule~~ **End** of ~~the~~ Study

Rationale: This section did not provide a clear definition of end of study.

Added a second paragraph:

The end of the study is defined as the date the last subject completes his final-visit 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.

Rationale: While this definition was also present in Section 7.1, it was not clearly defined here.

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.
- Sponsor Information was updated.
- Safety reporting email address was updated
- List of Abbreviations was updated.
- Typographical errors and formatting were corrected.
- Genome particles (gp) as a unit of measure was updated to vector genomes (vg).
- Reference list has been updated
- Footnotes in Table of Procedure updated

LIST OF ABBREVIATIONS

Abbreviation or Term	Definition
AAV	adeno-associated virus
AAV8-RPGR	AAV8 virus particle encapsulating 3.46 kb cDNA for the coRPGR gene
AE	adverse event
BCVA	best-corrected visual acuity
BGH	bovine growth hormone
BSS	balanced salt solution
cDNA	complementary deoxyribonucleic acid
CI	confidence interval
coRPGR	codon optimised human cDNA encoding RPGR
COVID-19	coronoavirus disease 2019
DLT	dose-limiting toxicity
DMC	Data Monitoring Committee
ERG	electroretinography
ET	early termination
ETDRS	Early Treatment of Diabetic Retinopathy Study
FDA	Food and Drug Administration
gp	genome particles
ICH	International Council for Harmonisation
IA	interim analysis
ITT	Intent to Treat
LLVA	low luminance visual acuity
LOCS III	lens opacities classification system III
MAIA	macular integrity assessment
MMRM	Mixed model repeated measures
RP	retinitis pigmentosa
RPGR	retinitis pigmentosa GTPase regulator
Rpgr	murine homologue of human RPGR
SAP	Statistical Analysis Plan
SAE	serious adverse event
VA	visual acuity
[redacted]	[redacted]
XLRP	X-linked retinitis pigmentosa

SUMMARY OF CHANGES

CLINICAL STUDY PROTOCOL
NSR-RPGR-01

AAV8-RPGR

A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

XIRIUS Study, Version 9 and Version 10

SPONSOR: NightstaRx Ltd
2nd Floor
10 Midford Place
London W1T 5BJ UK
Telephone: +44 (0) 1628 501000

ORIGINAL PROTOCOL DATE:	27 Sep 2016
VERSION 2:	01 Dec 2016
VERSION 3:	26 May 2017
VERSION 4:	15 Dec 2017
VERSION 5:	16 Jan 2018
VERSION 6:	18 MAY 2018
VERSION 7:	16 Nov 2018
VERSION 7.1	10 Dec 2018 (Administrative Amendment)
VERSION 8.0	18 Dec 2018
VERSION 8.1	08 Mar 2019 (Administrative Amendment)
VERSION 9.0	14 Aug 2019
VERSION 10	10 October 2019

SUMMARY OF CHANGES
SPONSOR APPROVAL PAGE

Clinical Study Protocol Number:	NSR-RPGR-01, Versions 9 and 10
Protocol Title:	A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)
Protocol Date:	10 Oct 2019
Approved By:	Ivana Vodopivec, MD Medical Director

The person listed above is authorised to sign the protocol on behalf of NightstaRx Ltd.

Signed: [redacted] Date: 10 Oct 2019

[redacted], MD

[redacted]

RATIONALE FOR SUBSTANTIAL AMENDMENT

PROTOCOL AMENDMENT XIRIUS VERSION 9.0

The most substantial change to the protocol is addition of an administrative interim analysis (IA) after all Part II subjects complete 3 months of post-treatment follow-up. This activity will enable a data readout and Phase 3 planning / regulatory interactions as discussed in prior regulatory agency meetings. Results of the interim analysis will then be presented to the regulatory agencies (both European Medicines Agency [EMA] and Food and Drug Administration [FDA]) for continued alignment on the acceptability of approach taken in evaluating the safety and efficacy of AAV8-RPGR.

Inclusion and exclusion criteria have also been modified.

- The requirement that subjects have a measurable ellipsoid zone (EZ) on optical coherence tomography (OCT) imaging has been removed to allow the broader study of X-linked retinitis pigmentosa (XLRP) subjects who do not have a measurable EZ.
- Documentation of a positive genetic screen now specifies that a **pathogenic** mutation must be present for clarification of test results from genetic screening.
- Best-corrected visual acuity (BCVA) now must achieve ≥34 letters in both eyes and not just the study eye to assure that no monocular or subjects with very poor vision in the fellow eye are enrolled.
- Exclusion has been added for subjects who are unsuitable candidates for surgery for any reason.

Study masking language has been improved and the specific assessments that must be taken in a masked fashion are better described. Also, the maintenance of masking of study teams during the interim analysis proceedings are described.

The assessment [redacted] has been removed from the study due to lack of operational feasibility.

Several changes to the Statistical Analysis and endpoints have been included:

- After meetings with both the EMA and FDA, the primary endpoint, microperimetry, has been clarified as a categorical endpoint that identifies the improvements in microperimetry at individual loci instead of overall mean retinal sensitivity. Therefore, the increase of ≥7 decibel (dB) in ≥5 central loci has been selected as the microperimetry endpoint. This same level of change in all 68 loci has become a new secondary endpoint. In agreement with the EMA, [redacted] have been de-ranked to exploratory endpoints.
- The safety analysis set has been clarified as including all treated subjects from both 2-arm and 3-arm randomization schedules, including subjects treated under previous versions of the protocol. However, the definition of the Intent to Treat (ITT) population has been clarified to include only 3-arm randomized subjects. The modified ITT population is also

described as the population of treated and not only randomized subjects; this population will be used for sensitivity and supportive analysis.
- Specific details of the efficacy analysis, including Berger-Boos correction, have been added now that the Statistical Analysis Plan has been completed, as well as clarifying why multiplicity adjustments are not needed.
- The administrative interim analysis is described.

Protocol template language regarding safety assessments has been updated. The definition of serious adverse events, and reporting of SAEs are better defined and consistent with other protocols. The definition, particularly regarding the timing, of vision loss post-treatment as an SAE is better defined.

The Data Monitoring Committee for Part II, which will participate in the interim analysis, is now described.

Additional minor changes are defined in the following Summary of Changes table.

RATIONALE FOR SUBSTANTIAL AMENDMENT

PROTOCOL AMENDMENT XIRIUS VERSION 10.0

In all previous versions of the protocol, a maximum time period of 8 weeks was allowed between the Baseline / Screening Visit and the day of surgery, Visit 2. This has been changed in Version 10 to a window of 12 weeks between screening and surgery in order to provide operational flexibility for the sites and investigators and to minimize patient burden for participation in the study. This change in no way affects the safety and efficacy of the drug, the outcomes of the study, as well as the ultimate analyses of the datasets.

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR) **Version 9.0** **Summary of Changes** **14 August 2019**			
Title Page	Telephone: ~~+44 (0) 207 062 2777~~1628 501000	Telephone: 1628 501000	New telephone number
Sponsor Approval, Page 2	[redacted]~~, MD~~ [redacted]	[redacted], MD [redacted]	New signatory
Contact Information Page 5	[redacted] ~~Serious adverse events, dose-limiting toxicities, and Pregnancy Notification Forms should be emailed to~~ [redacted] ~~For further information, refer to Section 12.0, Assessment of Safety.~~	Additional contact information is available in the Site Operations Manual.	Protocol Template language has been changed across all studies and protocols.
Synopsis Part I Secondary Endpoints Page 6		Part I Secondary Endpoints: 1, 3, 6, 9, 12, 18 and 24 months	Added 1 and 9 month posttreatment to assessment endpoints
Synopsis Part II Primary Endpoint Page 6	Part II ~~The primary efficacy endpoint is improvement from Baseline in microperimetry at 3 months.~~	The primary efficacy endpoint is the proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci of the 10-2 grid assessed by Macular Integrity Assessment (MAIA) microperimetry at 12 months.	Newly defined primary endpoint in alignment with feedback from regulatory agencies
Synopsis Part II		at 1, 2, 3, 6, 9, and 12 months	Added post-treatment time point assessments

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
Secondary Endpoints Page 6			to secondary endpoints
		• Proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of 16 central loci of the 10-2 grid assessed by MAIA microperimetry at 1, 2, 3, 6, and 9 months • Proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of 68 loci of the 10-2 grid assessed by MAIA microperimetry at 1, 2, 3, 6, 9, and 12 months	Added secondary endpoints in line with primary endpoint
	~~Change from baseline in SD-OCT at 3, 6, and12 months~~ ~~Change from baseline in fundus autofluorescence at 3, 6, and 12 months~~		Removed these as secondary endpoints, in agreement with EMA
Synopsis Part II Exploratory Endpoints Page 6	[redacted]		[redacted]
		[redacted]	Added as exploratory instead of secondary endpoints, in agreement with EMA
		[redacted]	
		[redacted]	[redacted]
		[redacted]	Added timepoints to exploratory endpoints

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
Synopsis Study Design Page 7	In Part II, all ~~subjects will be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), placed behind the globe via a deep sub-Tenon approach. All~~ subjects will also be given a 9-week course of oral corticosteroid starting 3 days before surgery: 21 days at 60 mg, followed by 6 weeks of tapering doses. The dose regimen is adjusted for pediatric subjects treated in Part II (see Section 9.8) .	In Part II, all adult subjects will be given a 9-week course of oral corticosteroid starting 3 days before surgery: 21 days at 60 mg, followed by 6 weeks of tapering doses. The dose regimen is adjusted for pediatric subjects treated in Part II (see Section 9.8) Subjects may also be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), administered via a deep sub-Tenon approach.	Clarified that triamcinolone is optional treatment
Synopsis Study Design Page 7-8		To further minimise potential bias of the treated and non-treated eye evaluations, all subjective ophthalmic assessments at the Screening/Baseline Visit (Visit 1) and from Month 3 (Visit 6) onwards will be conducted by an assessor who is masked to treated eye and treated vs. untreated control group.	Clarified that masking is to both treatment group and eye treated
		After all Part II subjects complete the 3-month visit, an administrative interim analysis (IA) will be conducted.	Added IA
		At study completion, treated subjects in Part II will be invited to participate in a separate long-term follow-up study that will collect efficacy and safety data up to 5 years from surgery.	Clarified that extension study is for treated subjects only
Synopsis Number of subjects planned Page 9		the study is expected to enroll approximately 63 subjects: 18 in Part I and up to 45 in Part II	Added flexibility in final sample size of Part II
Synopsis		Subject / parent / legal guardian (if applicable) is	Added legal guardian and

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
Inclusion Criteria Part II Page 9		willing and able to provide informed consent/assent for participation in the study	assent language for minors
	Documentation of a ~~non-synonymous~~ mutation in the RPGR gene	Documentation of a pathogenic mutation in the RPGR gene	Clarified genetic testing
	Have a BCVA ~~in the study eye~~ that meets the following criteria:	Have a BCVA in both eyes that meets the following criteria:	To prevent poor fellow-eye vision or monocular subjects from enrolling
Synopsis Exclusion Criteria Page 9		Are unwilling to use barrier contraception methods (if applicable), or abstain from sexual intercourse, for a period of 3 months	Added clarification
		c. unsuitability for retinal surgery	Added subsection c to list of general exclusions so to prevent subjects who are unsuitable candidates for surgery from enrolling
Synopsis Criteria for Evaluation Page 10	[redacted]		[redacted]
Synopsis Statistical Methodology Page 10		After all subjects in Part II complete the 3-month visit, an administrative IA will be conducted. The study will continue as planned. A final analysis will be conducted after all subjects have completed their final visit or are lost to follow-up.	Added IA to enable End of Phase 2 interactions with regulators and phase 3 planning as discussed with regulatory agencies
	~~Fisher's exact test will be utilized for the comparisons of binary endpoints, and T-test will be~~	The proportion of eyes with improved retinal sensitivity will be compared between study arms (high dose vs	Added statistical analysis of new primary endpoint; removed previous

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
	~~employed for the comparisons of continuous endpoints.~~	untreated; low dose vs untreated) using the Fisher Exact-Boschloo test with a Berger-Boos correction of beta=0.001 (Berger 1994). In addition, the difference in proportions between study arms will be presented with its corresponding 95% CI calculated using the method of Miettinen and Nurminen (Miettinen 1985). Change from baseline in mean sensitivity will be compared between study arms using an ANCOVA model including baseline value and study arm (high dose, low-dose, and untreated) as covariates. The difference in means between study arms, and its 95% CI, will be derived from the same ANCOVA model.	language regarding Fisher's exact test.
Abbreviations and Definitions Page 16	[redacted]		[redacted]
		EZ ellipsoid zone	Added EZ
Section 5.0 Introduction 5.1 X-Linked Retinitis Pigmentosa Page 18			Minor changes to text for clarification
Section 6.2.1.2 Part I Secondary Endpoints Page 22		Part I Secondary Endpoints: 1, 3, 6, 9, 12, 18 and 24 months	Added 1 and 9 month posttreatment to assessment endpoints
Section 6.2.2.1 Part II Primary Efficacy Endpoint Page 22	Part II ~~The primary efficacy endpoint is improvement from Baseline in microperimetry at 3 months.~~	The primary efficacy endpoint is the proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of the 16 central loci of the 10-2 grid assessed by Macular Integrity Assessment (MAIA) microperimetry at 12 months.	Newly defined primary endpoint in alignment with regulators

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
Section 6.2.2.3 Part II Secondary Endpoints Page 22		at 1, 2, 3, 6, 9, and 12 months	Added post-treatment time point assessments to secondary endpoints
	~~Change from baseline in SD-OCT at 3, 6, and 12 months~~ ~~Change from baseline in fundus autofluorescence at 3, 6, and 12 months~~	• Proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of 16 central loci of the 10-2 grid assessed by MAIA microperimetry at 1, 2, 3, 6, and 9 months • Proportion of study eyes with ≥7 dB improvement from baseline at ≥5 of 68 loci of the 10-2 grid assessed by MAIA microperimetry at 1, 2, 3, 6, 9, and 12 months	Added secondary endpoint in line with primary endpoint deranked [redacted] to exploratory in agreement with EMA
Section 6.2.2.4 Exploratory Endpoints Page 23	[redacted]		[redacted]
		[redacted]	Added to exploratory, removed from secondary
		[redacted]	[redacted]
		[redacted]	Added timepoints to exploratory endpoints
Section 7.1 Overall Study Design Page 24		Under version 6 of the protocol, Part II subjects were to be randomised into 2 active groups with a 2:1 allocation ratio (high dose [2.5 × 10^11	Added language on how the subjects randomized under previous versions

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
		gp] : low dose [5 × 10^10 gp]). Any subject randomised under this version will be followed for 12 months with the ongoing visit schedule and the data will be analysed separately (i.e., included in the Safety Analysis Set but not in the Intent to Treat Set, See Section 13.3).	of the protocol will be followed
		At study completion, all Part I subjects and treated subjects in Part II will be invited to participate in a long-term follow-up study that will collect efficacy and safety data up to 5 years from surgery.	Clarified that only treated subjects will be followed in extension study
		Once a subject has been randomised, a change in "study eye" designation is not permitted.	Added clarification not permitting change in study eye
	[redacted]	[redacted]	[redacted]
		After all Part II subjects complete the 3-month visit, an administrative interim analysis (IA) will be conducted. See Section 13.8 for further details.	Added description of IA
Section 7.1.3 Part II Dose Expansion, Version 6		Part II Dose-Expansion, Version 6 Under version 6 of the protocol, Part II subjects were to be randomised into 2 active groups with a 2:1 allocation ratio (high dose [2.5 × 10^11 gp] : low dose [5 × 10^10 gp]). Any subject randomised under this version will be followed for 12 months with the ongoing visit schedule and the data will be analysed separately (i.e., included in	Added section on follow-up of subjects from version 6 of protocol

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
		the Safety Analysis Set but not in the Intent to Treat Set, See Section 13.3). Enrollment under Version 6.0 has been superseded by the 3-arm randomisation ongoing since Version 7.	
Section 7.1.4 Part II Dose Expansion, Version 9.0		After all Part II subjects complete the 3-month visit, an administrative IA will be conducted. The study will continue as planned. A final analysis will be conducted at the end of the study, i.e., after all subjects complete the study. The end of the study is defined as the date the last subject completes his final-visit 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. See Section 13.8 for further details.	Added description of IA; clarified the end of study definition
Section 7.2 Number of Subjects Page 28		Overall, the study is expected to enroll approximately 63 subjects: 18 in Part I and up to 45 in Part II.	Updated sample size to allow for flexibility in final sample size of Part II
Part 8.2.1 Inclusion Criteria Page 30		Subject / parent / legal guardian (if applicable) is willing and able to provide informed consent/assent for participation in the study	Added legal guardian and assent for minors
	Documentation of a ~~non-synonymous~~ mutation in the RPGR gene	Documentation of a pathogenic mutation in the RPGR gene	Clarified genetic testing
	Have a BCVA ~~in the study eye~~ that meets the following criteria:	Have a BCVA in both eyes that meets the following criteria:	To prevent poor fellow-eye vision or monocular subjects from enrolling

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
Section 8.3 Exclusion Criteria, Parts I and II Page 31		Are unwilling to use barrier contraception methods (if applicable), or abstain from sexual intercourse, for a period of 3 months	Added to clarify contraception not needed if abstain from sexual intercourse
		c. unsuitability for retinal surgery	Added subsection c to list of general exclusions so to prevent subjects who are unsuitable candidates for surgery from enrolling
Section 8.4 Subjects Withdrawal Criteria Page 31	~~Subjects withdrawn from the MTD cohort may possibly be replaced.~~		Removed this sentence as this is no longer allowed
Section 9.4 Vitrectomy Procedure Pages 33-34	To date, over ~~150~~ subjects have been injected	To date, over 200 subjects have been injected	Updated number of subjects treated overall with this type of surgical procedure
	~~If deemed easier,~~ prior to sub-retinal injection of AAV8-RPGR, the retina will be detached		Optionality removed because all surgeries are required to do the 2-step BSS bleb
Figure 2 footnote Page 34	injection of BSS ~~if necessary~~, an		
Section 9.5 Randomisation Page 35		Once a subject has been randomized, a change in "study eye" designation is not permitted.	Added this requirement to not change study eye once selected
Section 9.6 Study Masking Page 35	In Part II, ~~all~~ ophthalmic assessments ~~that are conducted at the Screening/Baseline Visit will be conducted by appropriately qualified masked assessors.~~	In Part II, all ophthalmic assessments that will be used as efficacy endpoints (BCVA, LLVA, microperimetry, contrast sensitivity and [redacted]) will be conducted by appropriately qualified masked assessors (see Table 1).	Clarified when and which assessments must be masked
		It is preferable that the same assessor who performed the	Added that screening does

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
		efficacy endpoint assessments at Screening/Baseline also perform the assessments during the masked period from Month 3 to Month 12.	not need to be masked, but should if possible be conducted by same masked assessor as that of Month 3 to Month 12
		Table 1. Masked Assessments of Efficacy Masked Assessments at Month 3, 6, 9 and 12 Post-Treatment with AAV8-RPGR ➢ Best-corrected visual acuity ➢ Low-luminance visual acuity ➢ Microperimetry ➢ Contrast sensitivity ➢ 25-Item Visual Function Questionnaire	Table of assessments masked has been added
		After all Part II subjects complete the 3-month visit, an administrative IA will be performed by an independent DMC, who will review the accrued safety and efficacy data. A small group of unmasked Sponsor personnel, who will not be involved in the conduct of the study moving forward, will have access to unmasked data for this administrative IA to enable regulatory interactions and planning for phase 3 initiation. The operations team, the investigators, medical monitor, and other personnel who will continue to be involved in the daily operation of the study, as well as the subjects, will continue to be masked to the subjects' dose level assignment and	Description of maintenance of masking throughout the IA has been added

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
		outcomes of the IA until study completion to minimise any operational bias. Furthermore, the clinical assessors will continue to be masked to the treatment assignment. The full details of masking are provided in the study Masking Plan.	
Section 9.8 Concomitant Therapy Page 36-37	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/Baseline Visit and updated at every study visit (including the ET Visit, if applicable). Concomitant medications (including prednisone/prednisolone) 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).	Throughout the study, subjects may be prescribed any concomitant medications, procedures and/ or treatments deemed necessary for the subjects' ongoing medical care. Details of medications, treatments and procedures will be collected at the Screening/Baseline Visit and updated at every study visit (including the ET Visit, if applicable). Concomitant medications (including prednisone/prednisolone), treatments and procedures taken/undergone 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 assessment (e.g., topical anaesthesia, dilating eye drops).	Clarified that all information regarding medications, treatments and procedures must be collected and not just medications and treatments
	In Part I, all subjects will be prescribed a 21-day course of oral prednisone/prednisolone following closely the 17-day protocol established in the ~~Philadelphia~~ AAV gene therapy clinical trial for treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy (Maguire et al., 2008), ~~except~~ allowing	In Part I, all subjects will be prescribed a 21-day course of oral prednisone/prednisolone following closely the 17-day protocol established in the voretigene neparvovec-rzyl AAV gene therapy clinical trial for treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy (Maguire et al., 2008), allowing…	Added the details regarding the voretigene trial instead of referring to the site location

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
		In Part II, all subjects will be prescribed a course of oral corticosteroids. In addition, at the time of surgery, subjects (adult and pediatric) may be treated with up to 1 mL of triamcinolone,…administered via a deep sub-Tenon approach.	Clarified that triamcinolone is optional
Section 9.9 Treatment Compliance Page 38		The exact volume injected at the time of surgery should be recorded on the eCRF.	Added to correspond with electronic case report form
Section 10.1 Visit 1 (Screening/Baseline Period Page 39	Visit 1 (Screening/Baseline ~~Visit~~)	Visit 1 (Screening/Baseline Period)	Clarifies that Visit 1 can occur over more than one day
		The subject or parent / legal guardian	Added legal guardian for minors
	~~For Part I,~~ RPGR gene mutation screen (only if not conducted previously); ~~for Part II, documentation of a non-synonymous mutation in the RPGR gene~~		Clarified that mutation screen is needed for both Parts, but can be done specifically for this study
	[redacted]		[redacted]
		Footnotes have been re-numbered due to re-ordering of list	
Visit 1 Footnotes		1. In Part II, it is preferable that the same masked assessor who performs these assessments (LLVA, BCVA, microperimetry, contrast sensitivity and [redacted]) during the masked period from Month 3 through Month	Added footnote regarding masking

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
		12 also perform these assessments at screening.	
		3. Assessments at screening collected in triplicate. To facilitate triplicate testing, the visit should be conducted over 2 days. Visual field and microperimetry outputs will be sent to a CRC for review. Data will be generated and collated within the CRC and exported to the Sponsor or designee. For microperimetry, the last assessment performed during screening will be used for assessing eligibility and will be defined as the baseline assessment.	Clarified that the last microperimetry assessment will be considered baseline
		or abstain from sexual intercourse	Added that subjects may also abstain from sexual intercourse in lieu of contraception
		Subjects may be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), administered via a deep sub-Tenon approach.	Clarified that triamcinolone is optional
		or abstain from sexual intercourse	Added that subjects may also abstain from sexual intercourse in lieu of contraception
Section 10.4 Visit 4 Page 42		Where applicable, subjects will be reminded of the requirement to use barrier contraception or abstain from sexual intercourse for a period of 3 months from the time of treatment.	Clarified that subjects should be reminded for first 3 months after treatment.
Section 10.5 Visit 5		Where applicable, subjects will be reminded of the requirement to use barrier	Clarified that subjects should be reminded for

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
Page 43		contraception or abstain from sexual intercourse for a period of 3 months from the time of treatment.	first 3 months after treatment.
Section 10.6 Visit 5.9 Page 43		Where applicable, subjects will be reminded of the requirement to use barrier contraception or abstain from sexual intercourse for a period of 3 months from the time of treatment.	Clarified that subjects should be reminded for first 3 months after treatment.
Section 10.7 Visit 6 Page 44 Footnotes		1. In Part II, at Month 3, LLVA, BCVA, microperimetry and the [redacted] will be conducted by qualified masked assessors.	Footnote added to clarify which assessments must be masked from Month 3 to Month 12
Section 10.8 Visit 7 Page 44	[redacted]		[redacted]
		1. In Part II, at Month 3, LLVA, BCVA, microperimetry and the [redacted] will be conducted by qualified masked assessors.	Footnote added to clarify which assessments must be masked from Month 3 to Month 12
Section 10.9 Visit 8 Page 45		1. In Part II, at Month 3, LLVA, BCVA, microperimetry and the [redacted] will be conducted by qualified masked assessors.	Footnote added to clarify which assessments must be masked from Month 3 to Month 12
Section 10.10 Visit 9 Page 45	[redacted]		[redacted]
		1. In Part II, at Month 3, LLVA, BCVA, microperimetry and the [redacted] will be conducted by qualified masked assessors.	Footnote added to clarify which assessments must be masked from Month 3 to Month 12

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
Section 10.13 Early Termination Visit Page 47-48	[redacted]		[redacted]
		1. In Part II, at Month 3, LLVA, BCVA, microperimetry and the [redacted] will be conducted by qualified masked assessors.	Footnote added to clarify which assessments must be masked from Month 3 to Month 12
Section 10.14 Unscheduled Visits Page 48		For unscheduled visits that occur during Part II, BCVA and microperimetry will be conducted by qualified masked assessors from Month 3 through Month 12.	Footnote added to clarify that assessments must be masked if unscheduled visit occurs from Month 3 to Month 12
Section 11.1 Best-Corrected Visual Acuity Page 49		In Part I, at the Screening/Baseline Visit, eyes will be eligible for the study if they:	Specified that these BCVA criteria were applicable only to Part I
		In Part II, at the Screening/Baseline Visit, eyes will be eligible for the study if they have a BCVA better then or equal to 34 ETDRS letters.	Part II BCVA eligibility at baseline was clarified
		In Part II, it is preferable that the same masked assessor who performs the BCVA assessments during the masked period from Month 3 through Month 12 also perform these assessments at screening.	Although screening does not have to be masked, this statement clarifies that it is preferable that the same assessor perform assessments at baseline screening and from Month 3 to Month 12
[redacted]	[redacted]		[redacted]

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
[redacted]	[redacted]		[redacted]
Section 11.4 MAIA Microperimetry Page 50		In Part II, it is preferable that the same masked assessor who performs the microperimetry assessments during the masked period from Month 3 through Month 12 also perform these assessments at screening.	Although screening does not have to be masked, this statement clarifies that it is preferable that the same assessor perform assessments at baseline screening and from Month 3 to Month 12
Section 11.6 Contrast Sensitivity Page 51		In Part II, it is preferable that the same masked assessor who performs the contrast sensitivity assessments during the masked period from Month 3 through Month 12 also perform these assessments at screening.	Although screening does not have to be masked, this statement clarifies that it is preferable that the same assessor perform assessments at baseline screening and from Month 3 to Month 12
Section 11.7 Low-luminance Visual Acuity Page 51		In Part II, it is preferable that the same masked assessor who performs the LLVA assessments during the masked period from Month 3 through Month 12 also perform these assessments at screening.	Although screening does not have to be masked, this statement clarifies that it is preferable that the same assessor

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
			perform assessments at baseline screening and from Month 3 to Month 12
[redacted]		[redacted]	[redacted]
Section 12 Throughout	~~medication~~	drug	In all the AE definitions section, the word 'medication' has been changed to 'drug' to align with all protocols
Section 12.2.1.2 Serious Adverse Event Page 53	Hospitalisation for a pre-existing condition, ~~including elective procedures,~~ which has not worsened, does not constitute an SAE.	Hospitalisation that was pre-scheduled or for an elective procedure or routinely scheduled treatment for a pre-existing condition, which has not worsened, does not constitute an SAE.	Clarified the types of hospitalization that would be defined as SAEs; made consistent template language across studies ad protocols
Section 12.2.1.2 Serious Adverse Event Page 54	~~The following vision loss or vision-threatening events are to be reported as SAEs:~~ ~~• Sustained decrease in VA of ≥15 letters on ETDRS chart compared to baseline, except for surgery-related events.~~ ~~Sustained is defined as lasting 48 hours or more until recovery;~~	Vision Loss to Be Reported as a Serious Adverse Event 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.	Clarified definitions for vision loss reporting as SAE

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
	~~recovery defined as VA returned to within 5 letters of baseline VA.~~ ~~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 (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 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: o Has an evolution not consistent with the expected post-operative course; o May be attributable to a complication that occurred during surgery, or another untoward event, or the study drug; o 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 loss is defined as a return to within 10 letters of the baseline BCVA on the ETDRS chart.	
Section 12.2.2 Recording of Adverse Events Page 54		/ legal guardian	Added legal guardian for minors
		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.	Updated text for time points, evaluation and definition of AE severity and relationship;

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
			clarified and updated to be consistent across all studies and protocols
		AE Severity… AE Relationship Unrelated: 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:… Definitely related: There is a causal …. When a relationship is determined to exist, the investigator or medical designee will further define if that relationship is to the study drug, the surgical procedure, both, or unknown.	Updated text for time points, evaluation and definition of AE severity and relationship Definitions of AE relationship are now binary (related / unrelated) with 3 possibilities of relatedness as a subcategorization. This eliminates the 'unlikely to be related' which is subject to error
Section 12.2.3 Follow-up of Adverse Events Page 56		/ legal guardian	Legal guardian added for minors
		Investigators are expected to timely provide the requested additional information for a complete assessment and documentation of the SAE reports in a timely manner.	Added clarification

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
Section 12.2.4 Reporting of Serious Adverse Events and Dose-Limiting Toxicities Page 56-57	emailing ~~a~~ DLT/SAE form ~~to~~ [redacted]	emailing the DLT/SAE form. For reporting purposes, the date of SAE form submission by the investigator to the Sponsor will be designated as Day 0.	Clarified the procedure and timing of SAE reporting.
		Follow-up SAE reports will be submitted within 15 days of receiving the information.	Clarified the procedure and timing of SAE reporting.
Section 12.2.6 Data Monitoring Committee Page 57		For Part I, an independent DMC	Clarified that the fist paragraph described the safety monitoring responsibilities of the Part I DNC only
		After all Part II subjects complete the 3-month visit, an administrative IA will be performed by an independent DMC, who will review the accrued safety and efficacy data. A small group of unmasked Sponsor personnel, who will not be involved in the conduct of the study moving forward, will have access to unmasked data for this administrative IA to enable regulatory interactions and planning for phase 3 initiation. Following the administrative IA, the DMC will continue to meet, as described in the DMC Charter.	Defined the activities of the Part II DMC
Section 12.3 Pregnancy Page 57	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 a Pregnancy Notification Form ~~to~~ [redacted] In addition, if possible, outcome of the	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)	Clarified that informed consent must first be obtained before collecting information from a potential pregnant partner

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
	pregnancy fathered by the subject should be recorded ~~and followed up until delivery for,~~ congenital abnormality or birth defects.	report the pregnancy, with at least preliminary data,… In addition, if possible, outcome of the pregnancy fathered by the subject should be recorded including any congenital abnormality or birth defects.	
Section 12.7.3 Immunogenicity Testing Page 59		Enzyme-linked immunospot (ELISPOT) assays will be used…. and cell-based methods.	Clarified the methodology and storage of samples for immunogeniciity
	All immunogenicity samples will be sent to ~~and stored at~~ a central ~~laboratory~~ laboratories for ~~future~~ analyses.	All immunogenicity samples will be sent to central laboratories for analyses.	
		Residual samples may be stored for up to 15 years or per local regulations.	
Section 13.3.1 Safety Analysis Set Page 60	The Safety Analysis Set will consist of all subjects who receive study treatment (vitrectomy/AAV8-RPGR) ~~in Part I, and all subjects that are randomized in Part II.~~	For Part I, the Safety Analysis Set will consist of all subjects who receive study treatment (vitrectomy/AAV8-RPGR).	Clarified that all subjects treated under 2-arm and 3-arm randomization schedules will be included in the Safety Analysis Set. This accounts for any subjects enrolled and treated under previous versions of the protocol.
		For Part II, the Safety Analysis Set will consist of all subjects who are randomised, under both the 2- and the 3-arm randomisation schedules. The Safety Analysis Set will be the primary population for demographics, baseline characteristics and safety analyses. Subjects will be analysed based on the actual treatment received.	
Section 13.3.2 Intent-to-Treat (ITT) Analysis Set Page 60	~~The Full Analysis Set will include all subjects for whom data of at least 1 post baseline efficacy assessment is available in the study~~	The intent-to-treat (ITT) Analysis Set will consist of all Part II subjects who are randomised, under the 3-arm randomisation schedules. The ITT Analysis Set will be used	The ITT set is now clarified to include only subjects treated under the 3-arm randomization

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
	~~eye. The Full Analysis Set will be used for efficacy analyses.~~	for the efficacy analyses. Subjects will be analysed based on the treatment to which they were randomised. The modified intent-to-treat (mITT) Analysis Set will include all Part II subjects who are randomised under the 3-arm randomisation schedule and who receive study treatment (or the phone call for those in the untreated control group) and who have baseline microperimetry data and at least 1 post-baseline microperimetry data in the study eye. The mITT Analysis Set will be used for supportive and sensitivity analysis for Part II primary and secondary efficacy endpoints. Subjects will be analysed based on the treatment to which they were randomised.	schedule. It also clarifies that the true definition of ITT will be used for the primary endpoint analysis. The modified ITT set will be used to analyse the primary and secondary endpoints in sensitivity and supportive analysis because some subjects might be randomized and not treated.
Section 13.6 Efficacy Analyses		The proportion of eyes with improved retinal sensitivity, for both the center grid (i.e., the central 16 loci) and the entire grid (i.e., all 68 loci), will be compared between study arms (high dose vs untreated; low dose vs untreated) using the Fisher Exact-Boschloo test with a Berger-Boos correction of beta=0.001 (Berger 1994). In addition, the difference in proportions between study arms will be presented with its corresponding 95% CI calculated using the method of Miettinen and Nurminen (Miettinen 1985). Change from baseline in mean sensitivity, in both the center grid and the entire grid, will be compared between study	The Statistical Analysis Plan has since been finalized and therefore further details on the statistical analyses have been added to the protocol

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
		arms using an ANCOVA model including baseline value and study arm (high dose, low-dose, and untreated) as covariates. The difference in means between study arms, and its 95% CI, will be derived from the same ANCOVA model.	
Section 13.6.1 Multiplicity Adjustment Page 61		In Part II, an administrative IA is included to facilitate the planning of Phase 3 activities. As no early stopping for efficacy is allowed, no multiplicity adjustment is required for the administrative IA. The final analysis will be conducted at 0.05 two-sided level for the primary endpoint.	It is clarified that multiplicity adjustment is not needed in conducting the administrative IA because no early stopping for efficacy is allowed.
Section 13.8 Interim Analysis Page 62		In Part II, after all subjects complete the 3-month visit, an administrative IA will be performed by an independent DMC. The DMC will review the accrued safety and efficacy data. A small group of unmasked Sponsor personnel, who will not be involved in the conduct of the study moving forward, will have access to unmasked data for this administrative IA to enable regulatory interactions and planning for phase 3 initiation. The details of the roles and responsibilities of the Part II DMC are provided in the DMC charter, and the full details of masking are provided in the study Masking Plan. The study will continue as planned. A final analysis will be conducted at the end of the study, i.e., after all subjects	Details of the administrative IA are included for Part II.

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
		complete the study. The end of the study is defined as the date the last subject completes his final-visit 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.	
Section 14.1 Informed Consent Page 64		/legal guardian	Legal guardian language is included throughout in the case of minors
		In the case of a female partner pregnancy fathered by a subject, a partner pregnancy informed consent form must be signed and dated before collecting information regarding the outcome of the pregnancy (birth or spontaneous abortion), as well as any incidents of congenital abnormality, birth defect or other medical problems.	The requirement for pregnant partner informed consent prior to obtaining any information is included
Section 14.2 Ethical/Regulatory Review Page 64		, in accordance with local requirements,	Clarified that submission is necessary in accordance with local requirements only
Section 14.3 Regulatory Considerations Page 65		The study will be conducted in accordance with the relevant articles of the Declaration of Helsinki, where permissible.	In some regions, the Declaration of Helsinki cannot be adhered to.
Section 15.2 Data Handling and Records Management Page 66		All study data will be entered on an encrypted EDC system with individual pass-codes known to all each investigators	Clarified the process of data handling

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
	~~Two reading centres are anticipated.~~		Removed unnecessary detail
Section 16 References Page 68		Berger R.L., Boos D.D. P values maximized over a confidence set for the nuisance parameter. Journal of the American Statistical Association 1994; 89: 1012-1016 Miettinen, O. S. and Nurminen, M. 'Comparative analysis of two rates', Statistics in Medicine, 4, 213-226 (1985).	Added reference related to the statistical tests used
Table 3 Page 73-76	[redacted]		[redacted]
		Reordered al footnote numbering	
Footnote l		The final microperimetry assessment will be used to determine eligibility.	Added a clarification on microperimetry that the final assessment should be used for baseline to determine eligibility
Throughout Protocol			Changed Years to months post-treatment for consistency

Section Changed	Original Text (crossed out)	Amended Text (in red)	Rationale
A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR) **Version 10.0** **Summary of Changes** **10 October 2019**			
All Headers	~~Version 9~~ ~~14 Aug 2019~~	Version 10.0 10 Oct 2019	Upversion and date change, throughout document
Title Page		Version 10.0 10 Oct 2019	Added version and date to list of amendments
Sponsor Approval Page, 3		Version 10.0 10 Oct 2019	Added version and date to list of amendments
Investigator's Signature Page Page 4		Version 10.0 10 Oct 2019	Added version and date to list of amendments
Section 10.1 Visit 1 (Screening/Baseline Period) Page 38	The next study visit (Visit 2) is to be scheduled within ~~8~~ weeks of the Screening/Baseline Visit.	The next study visit (Visit 2) is to be scheduled within 12 weeks of the Screening/Baseline Visit.	Window changed from 8 to 12 weeks between screening and surgery to provide increased operational flexibility and decrease patient burden
Table 3, Schedule of Study Procedures, Footnote a, Page 72	The Screening/Baseline Visit must be performed within ~~8~~ weeks of Visit 2	The Screening/Baseline Visit must be performed within 12 weeks of Visit 2	See above

SUMMARY OF CHANGES FOR CLINICAL STUDY PROTOCOL NSR-RPGR-01

Version 6.0 to Version 8.0

AAV8-RPGR

A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

Indication:	X-Linked Retinitis Pigmentosa
Study Phase:	1/2/3
Sponsor:	NightstaRx Ltd 2nd Floor 10 Midford Place London W1T 5BJ, UK Telephone: +44 (0) 020 7062 2777
Summary of Changes:	Protocol Version 6.0 to 8.0 18 DEC 2018

CONFIDENTIALITY STATEMENT

SUMMARY OF CHANGES SPONSOR APPROVAL PAGE

Clinical Study Protocol Number:	NSR-RPGR-01
Protocol Title:	A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)
Summary of Changes for Protocol:	Version 6.0 to 8.0 18 DEC 2018

Approved By:

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

[redacted] Date: 19 December 2018

[redacted] MD

Overview / Rationale: Changes to the Part II Dose-Expansion Phase of the Study

From NSR-RPGR-01 Version 6.0 to 7.0, the protocol amendment changes the doses to be expanded in Part II from the "MTD and 3-dose levels lower than MTD" to dose 3 and dose 5. Rationale for this change was provided by the DMC at the October 2018 meeting. Although no DLTs have been identified through cohort 6 treated with the highest dose, and therefore a true MTD has not been identified in this dose-escalation, the committee recommended to expand dose 5 and dose 3, as the doses most likely to define safety and efficacy of AAV8-RPGR. In addition, regulatory agencies have recommended adding a third untreated arm to better establish efficacy and safety of the gene therapy. Thus, the subjects will be randomized in a 1:1:1 allocation, with double masking to dose in the treated arms, and masked assessments of efficacy.

The study duration has been shortened to 12 months of follow-up for Part II dose-expansion because a long-term follow-up study will be initiated to continue follow-up of all AAV8-RPGR subjects from 12 months to out to 5 years. Phase 1 subjects will still be followed for 24 months, and then also invited to participate in the long-term study.

The endpoints and inclusion criteria are now separated by study Part to improve clarity.

An efficacy endpoint, microperimetry at 3 months, has been added to Part II based on early efficacy signals observed during dose-escalation. Related to this, an inclusion range for microperimetry has been added to Part II in order to assure the inclusion of subjects with modifiable disease and to avoid inclusion of those with microperimetry values subject to ceiling effects.

Additionally, to further our understanding of the effects of AAV8-RPGR, assessments of [redacted], [redacted] and quality of life questionnaires have been added, the former two only in sites with the available technology. Some assessments (reading test, color vision, and full-field threshold sensitivity [FST]) have been removed from the study assessments due to the excessive burden of study visits on the subjects, and the limited value of these assessments in understanding both the disease and the effects of the drug.

To improve safety and to prevent potential inflammatory response to therapy, the steroid regimen has been modified. A standardized adult starting dose of 60 mg has been selected (rather than the previous 1mg/kg starting dose) and the taper has been lengthened to include coverage out to approximately 2 months. A 2-month visit has been added to assess the subjects at the end of the steroid therapy.

Information on the procedures related to unmasking has been added to comply with Good Clinical practices. Serious adverse event reporting has also been changed to immediately instead of within 7 days, per regional recommendations.

From NSR-RPGR Version 7.0 to Version 7.1, this administrative protocol amendment clarifies the procedures and requirements for assessments done at each visit compared to the Schedule of Study Procedures. Footnotes for BCVA assessment, Immunogenicity sampling, and corticosteroid review were added to the in-text footnotes and Schedule of Study Procedures. In addition, Fundus photography was removed from Visit 6 and Visit 7, viral shedding was removed from Visit 6, and autofluorescence assessment was added to the Unscheduled Visit. Updates were made to the protocol to accommodate these changes.

Page numbers on the following table of changes refer to the tracked changes version of the protocol amendment.

From Version 7.1 to Version 8.0, the unmasking information has been modified in Section 12.2.5 to adhere more closely to Good Clinical Practices. Also, the mobility test has been added to the Early termination Visit list of procedures, to be conducted if feasible.

Updates noted in italics are those referring to Version 7.0 to Version 7.1.

Updates noted in italics and bold, with grey shading, are those referring to Version 7.1 to Version 8.0.

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Title Page	**A Dose Escalation, Phase ~~1/2~~ Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)**	A Dose Escalation (Phase 1), and Dose-Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)	Added to title the dose-expansion and phase of the study has been specified for dose escalation to be Phase 1 and for dose expansion, to be Phase 2/3
Title Page, Header		*Version 7.1* *07 Dec*	*Updated Header*
Title Page, Header		***Version 8.0*** ***18 Dec 2018***	***Updated Header***
Title Page, Pages 1, 5, 6, 22		STUDY PHASE 1/2/3	Added Phase 3 to Study Phase
Title Page, Original Protocol Date		*VERSION 7.1 07 Dec 2018*	*Added Version 7.1*
Title Page, Original Protocol Date		***VERSION 8.0 18 Dec 2018***	***Added Version 8.0***
Sponsor. Page 1	~~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	[redacted]	[redacted], MD	Personnel change
	~~16 Nov~~	*07 Dec*	*Updated date for version 7.1*
		18 Dec 2018	***Updated date for version 8.0***
Investigator's Signature Page	~~16 Nov~~	*07 Dec*	*Updated date for version 7.1*
		18 Dec 2018	***Updated date for version 8.0***
Contact Information. Pages 4	[redacted] ~~Rx Ltdr Wellcome Gibbs Building, Euston Road London NW1 2BE, UK +44 (0) 207 611 2034~~		The contact page within the protocol now includes only the pharmacovigilance contact and the responsible CRO. The complete study
	[redacted]		

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~NightstaRx Ltd Wellcome Gibbs Building, 215 Euston Road London NW1 2BE, UK +44 (0) 207 611 2193~~		contact list will be included in the study manual. The safety email remains the same and is listed.
	~~[redacted], MD NightstaRx Ltd Wellcome Gibbs Building, 215 Euston Road London NW1 2BE, UK~~	[redacted] Email: [redacted] +1-919-401-8003	Change in personnel and contact information
	Contract Research Organization [redacted] ~~UK~~	[redacted] UK	New address and phone
	~~[redacted] NightstaRx Ltd Wellcome Gibbs Building, 215 Euston Road London NW1 2BE, UK 781 457 +44 (0) 207 611 2271~~		The contact sheet within the protocol now includes only the pharmacovigilance contact and the responsible CRO. The complete study contact list will be included in the study manual. The safety email remains the same and is listed.
	~~81 Hatrwell Avenue, Suite 100 Lexington, MA 02421~~		
Synopsis Study Period (years) Page 5	~~36~~ months	Part I: 24 months Part II: 12 months	Duration of study corrected from estimate last patient, last visit to actual duration for each subject in Part I
Synopsis Inclusion Criteria Page 8	*~~Prior~~ documentation…*	*Documentation*	*Clarified documentation requirement*
Synopsis Endpoints Page 5	~~The primary safety endpoint is incidence of dose-limiting toxicities (DLTs) and treatment-emergent adverse events~~	**Endpoints Part I Primary Endpoints:** The primary safety endpoints are the incidence of dose-limiting toxicities (DLTs), and treatment-emergent	Separated out Part I

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~(TEAEs) over a 24-month period.~~	adverse events (TEAEs) over a 24-month period. Secondary and Exploratory Endpoints: • Change from baseline in microperimetry at 3, 6, 12, 18 and 24 months • Change from baseline in best-corrected visual acuity (BCVA) at 3, 6, 12, 18, and 24 months • Change from baseline in spectral domain optical coherence tomography (SD-OCT) at 3, 6,12, 18 and 24 months • Change from baseline in autofluorescence at 3, 6, 12, 18 and 24 months • Change from baseline in other anatomical and functional outcomes at 3, 6, 12 months, 18 and 24 months	
Synopsis. Endpoints Pages 5-6		**Part II** **Primary Efficacy Endpoint:** The primary efficacy endpoint is improvement from Baseline in microperimetry at 3 months. **Safety Endpoint:** The safety endpoint is incidence of TEAEs over a 12-month period.	Separated out Part II
Synopsis. Endpoints Pages 5-6	• Change from baseline in microperimetry at ~~3,~~ 6, 9, 12~~-18 and 24~~ months • Change from baseline in best-corrected visual acuity (BCVA) at 3, 6, 12~~-18 and 24-~~months • Change from baseline in SD-OCT at 3, 6, 12 ~~18 and 24-~~months • Change from baseline in autofluorescence at 3, 6, 12,~~-18 and 24~~ months	**Secondary Endpoints:** • Change from baseline in microperimetry at 1, 6, 9, and 12 months • Change from baseline in best-corrected visual acuity (BCVA) at 3, 6, and 12 months • Change from baseline in SD-OCT at 3, 6, and 12 months • Change from baseline in autofluorescence at 3, 6, and 12 months • Change from baseline in visual field assessed by Octopus 900 at 3, 6, and 12 months	Updated Part II endpoints
	• Change from baseline in other anatomical	Exploratory Endpoints:	• Updated Part II endpoints

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	and functional outcomes at 3, 6, 12, ~~18 and 24~~ months	• [redacted] • [redacted] • [redacted] • [redacted]	
Synopsis. Study Design Pages 6-7	~~The study will be conducted in two parts: Part I is a dose escalation study, Part II is a Maximum Tolerated Dose (MTD) expansion study (as determined in Part I)~~	This is a Phase 1/2/3… Part I is a dose-selection study; Part II is a dose-expansion study, with 2 doses (2.5 × 10^11 gp [high dose], 5 × 10^10 gp [low dose]) selected from Part I based on a benefit/risk assessment, and a third untreated group to allow for a controlled comparison of efficacy and safety.	Study design description updated to include Part I and Part II. Part II now defines doses as high and low doses and not as MTD since the chosen high dose to be expanded has not been defined as MTD.
	The study will consist of visits over a 24-month evaluation period. At the Screening/Baseline Visit, each subject will be assessed for eligibility of both eyes. Only 1 eye will ~~receive treatment~~ (the "study eye"), and the ~~untreated~~ eye will be designated as the "fellow eye".	Part I consists of 12 visits over a 24-month evaluation period. Part II consists of 10 visits over a 12-month evaluation period. Only 1 eye will be randomized (the "study eye"), and the other eye will be designated as the "fellow eye". Selection of the "study eye" will be made on clinical grounds prior to randomization and will generally be the worse eye affected.	Differentiates the visit number and duration for Parts I and II
		Subjects in the untreated control group will receive study-visit telephone calls to monitor AEs/SAEs and review concomitant medications on Visit 2 (Day 0) and on Visits 3 and 4 (post-operative Days 1 and 7).	Information on conduct for untreated control subjects has been added regarding the surgery day and post-operative Days 1 and 7.
	To minimise inflammation resulting	To minimise inflammation resulting from surgery and/or	To improve safety and to prevent

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	from surgery and/or vector/transgene, all subjects will be given a 21-day course of oral corticosteroid (e.g., prednisolone/prednisone) that will start 2 days before the planned date of surgery.	vector/transgene, in Part I, all subjects will be given a 21-day course of oral corticosteroid (e.g., prednisolone/prednisone) that will start 2 days before the planned date of surgery. In Part II, all subjects will be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), placed behind the globe via a deep sub-Tenon approach. All subjects will also be given a 9-week course of oral corticosteroid starting 3 days before surgery: 21 days at 60 mg, followed by 6 weeks of tapering doses. The dose regimen is adjusted for pediatric subjects treated in Part II (see Section 9.8).	potential inflammatory response to therapy, the steroid regimen has been modified. A standardized adult starting dose of 60 mg has been selected (rather than the previous 1mg/kg starting dose) and the taper has been lengthened to include coverage out to approximately 2 months. A 2-month visit has been added to assess the subjects at the end of the steroid therapy.
		At study completion, Part I subjects will be invited to participate in a separate long-term follow-up study that will collect efficacy and safety data up to 5 years from surgery.	Added the extension of follow-up for Part I subjects
	Part II: ~~MTD~~ Expansion ~~Study~~	Part II: Dose Expansion In Part II, 45 subjects will be randomized 1:1:1 to a high-dose (2.5 × 10^11 gp), a low-dose (5 × 10^10 gp), and a third untreated group to allow for a controlled comparison of efficacy and safety. Study data will be collected for both eyes of each subject. Since treatment requires an invasive surgical procedure under general anaesthesia, the sponsor, investigator and the subject will be unmasked to the study procedure (i.e., vitrectomy and sub-retinal injection), however within the treated groups, the sponsor, investigator and subject will be masked to the assigned dose. To further minimise potential bias of the treated and non-treated eye evaluations, all subjective	Clarified randomization for Part II Dose Expansion as 3-armed study with untreated arm added, per general rationale above. Added language explaining the assessor masking.

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		ophthalmic assessments at the Screening/Baseline Visit (Visit 1) and from Month 1 (Visit 5) onwards will be conducted by a masked assessor.	
	~~Once the MTD has been identified, up to 45 additional subjects will be randomized, in a 2:1 allocation ratio. Subjects will receive AAV8-RPGR either at the MTD (MTD cohort), or at a low dose (active control cohort), three dose-levels below the MTD (e.g., low dose = 5 x 1010 gp if MTD = 5x1011 gp). Part II of the study will be randomized and double-masked to the assigned dose, and open-label to the treatment administration.~~	Subjects, sponsor, investigators and clinical assessors will be masked to the assigned dose. All Part II subjects will be followed for 12 months with the pre-specified visit schedule. At study completion, Part II subjects will be invited to participate in a separate long-term follow-up study that will collect efficacy and safety data from Month 12 through 5 years from surgery.	Clarified randomization for Part II Dose Expansion as 3-armed study with untreated arm added, per general rationale above.
Synopsis Inclusion Criteria Page 7	1. Subject~~/ parent (if applicable)~~ is willing and able to give informed consent for participation in the study		Part I is only in adults so removed the language for minors
Synopsis Inclusion Criteria Page 7	2. Are male and able to comply and adequately perform all study assessments ~~Part I:≥18 years of age~~ ~~Part II: ≥10 years of age~~	Part I Are male, ≥18 years of age, and able to comply and adequately perform all study assessments.	Clarified age inclusion criterion for Part I; separated Part I and Part II inclusion criteria to add clarity for investigators;
Synopsis Inclusion Criteria Page 7	5. BCVA in both eyes that meets the following criteria, based on the cohort level: Cohort 4-6~~ and study Part II~~: better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity)	5. BCVA in both eyes that meets the following criteria, based on the cohort level: Cohort 4-6: better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity)	Removed Part II BCVA inclusion criterion because inclusion criteria are now separated for Part II to improve clarity

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Synopsis Inclusion Criteria Part II Pages 8		Part II Inclusion Criteria 1. Subject / parent (if applicable) is willing and able to provide informed consent for participation in the study 2. Are male, ≥10 years of age, and able to comply and adequately perform all study assessments 3. Documentation of a non-synonymous mutation in the RPGR gene 4. Have active disease clinically visible within the macular region in the study eye and defined as follows: ➢ ellipsoid zone (EZ) on SD-OCT at screening must be measurable, and within the nasal and temporal border of any B-scan, and not be visible on the most inferior and superior B-scan 5.Have a BCVA in the study eye that meets the following criteria: •Better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity). 6. Mean total retinal sensitivity in the study eye as assessed by microperimetry ≥0.1 dB and ≤8 dB	• Added section for Part II inclusion criteria to clarify the differences between Parts I and II; • EZ zone must be measurable on screening in the study eye • BCVA is same for all subjects in Part II (no cohort differences); specifies criterion is for study eye only • Added mean total retinal sensitivity range in the study eye to ensure that subjects have modifiable microperimetry not subject to ceiling effects
Synopsis, Exclusion Criteria Page 9		Exclusion Criteria: Parts I and II	Specifies that exclusion criteria are the same for both parts
Synopsis Test Product, Dosage, and Mode of Administration Page 9		Part I, Dose Escalation, Phase 1: All subjects will undergo vitrectomy and receive a single sub-retinal injection of AAV8-RPGR. Subjects will be assigned to 1 of the following AAV8-RPGR dose levels: 5 × 10^9 gp, 1 × 10^10 gp, 5 × 10^10 gp, 1 × 10^11 gp, 2.5 × 10^11 gp, or 5 × 10^11 gp. Part II, Dose Expansion, Phase 2/3: Subjects will be assigned to 1 of the following: high-dose (2.5 × 10^11 gp), low-dose (5 × 10^10 gp), or an untreated control arm.	Separated out Parts I and II for improved clarity

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Synopsis. Reference Therapy Page 10	In Part I, no Reference therapies will be administered. In Part II, ~~a high-dose and a low-dose of AAV8-RPGR (active-control cohort) the concentration of which will be three dose-levels from MTD.~~	In Part I, no Reference therapies will be administered. In Part II, 2 doses of AAV8-RPGR will be compared: a high-dose (2.5 × 10^11 gp) and a low-dose (5 × 10^10 gp). An untreated control arm will also be added.	Added comparator dosing and untreated arm to reference therapy
Synopsis. Criteria for Evaluation Page 10	**Efficacy:** The efficacy evaluation will be based on BCVA, SD-OCT, fundus autofluorescence, visual fields, microperimetry, contrast sensitivity, low luminance VA, ~~full field stimulus threshold test, colour vision and reading test.~~	**Efficacy:** The efficacy evaluation will be based on microperimetry (MAIA), BCVA, SD-OCT, [redacted], fundus autofluorescence, visual fields (Octopus 900 specified in Part II only), contrast sensitivity, LLVA, [redacted]	Efficacy evaluation updated to include MAIA microperimetry; for Part II only, [redacted] have been added; specifies Octopus 900 for visual field only in Part II
Synopsis Statistical Methodology Page 10	The primary objective is to evaluate…	The primary objective of Part I is to evaluate…	Differentiates Part I objective from Part II objective
		The primary objective of Part II is to evaluate the efficacy and safety of AAV8-RPGR in an expanded population of subjects.	Differentiates Part I objective from Part II objective
	~~A sample size of 30 subjects at the MTD dose ensures that events with an incidence ≥10% will be identified with a 95% probability.~~	In Part II, subjects will be randomized 1:1:1 to the 2 doses of AAV8-RPGR: 2.5 × 10^11 gp and 5 × 10^10 gp or an untreated control group.	Randomization clarification for Part II, with addition of untreated control group
		Fisher's exact test will be utilized for the comparisons of binary endpoints, and T-test will be employed for the comparisons of continuous endpoints.	Statistical methods to include Fisher's exact test and T-tests
Section 6 Study Objectives and Endpoints Page 20	~~The primary safety endpoint is incidence of dose-limiting toxicities (DLTs) and treatment-emergent adverse events (TEAEs) over a 24-month period.~~	**Endpoints Part I Primary Endpoints:** The primary safety endpoints are the incidence of dose-limiting toxicities (DLTs), and treatment-emergent adverse events (TEAEs) over a 24-month period. Secondary and Exploratory Endpoints:	Separated out Part I

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		• Change from baseline in microperimetry at 3, 6, 12, 18 and 24 months • Change from baseline in best-corrected visual acuity (BCVA) at 3, 6, 12, 18, and 24 months • Change from baseline in spectral domain optical coherence tomography (SD-OCT) at 3, 6,12, 18 and 24 months • Change from baseline in autofluorescence at 3, 6, 12, 18 and 24 months • Change from baseline in other anatomical and functional outcomes at 3, 6, 12 months, 18 and 24 months	
Section 6.2.2 Study Objectives and Endpoints Page 20		**Part II Primary Efficacy Endpoint:** The primary efficacy endpoint is improvement from Baseline in microperimetry at 3 months. **Safety Endpoint:** The safety endpoint is incidence of TEAEs over a 12-month period.	Separated out Part II
Section 6.2.2.3 Study Objectives and Endpoints Page 20-21	• Change from baseline in microperimetry at ~~3,~~ 6, 9, 12~~ 18 and 24~~ months • Change from baseline in best-corrected visual acuity (BCVA) at 3, 6, 12~~ 18 and 24~~ months • Change from baseline in SD-OCT at 3, 6, 12 ~~18 and 24~~ months • Change from baseline in autofluorescence at 3, 6, 12,~~ 18 and 24~~ months	**Secondary Endpoints:** • Change from baseline in microperimetry at 1, 6, 9, and 12 months • Change from baseline in best-corrected visual acuity (BCVA) at 3, 6, and 12 months • Change from baseline in SD-OCT at 3, 6, and 12 months • Change from baseline in autofluorescence at 3, 6, and 12 months • Change from baseline in visual field assessed by Octopus 900 at 3, 6, and 12 months	Updated Part II endpoints

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	• Change from baseline in other anatomical and functional outcomes at 3, 6, 12, ~~18 and 24~~ months	Exploratory Endpoints: [redacted]	Updated Part II endpoints
Section 7.1. Overall Design Pages 22-25	~~Part II is a Maximum Tolerated Dose (MTD) expansion study (as determined in Part I).~~	This is a Phase 1/2/3… The study will be conducted in two parts: Part I is a dose escalation study, Part II is a dose-expansion study, with 2 doses selected from Part I based on safety and efficacy, and a third untreated group to allow for a controlled comparison of efficacy. Part I will identify the maximum tolerated dose (MTD) using a dose-escalation scheme. Part II will expand 2 doses, allowing for a broader assessment of the safety and efficacy of AAV8-RPGR with a larger sample size, including 45 subjects randomized 1:1:1 to the a high-dose, a low-dose, and an untreated arm. Part I primarily evaluates safety, defined by incidence of DLTs and TEAEs over a 24-month period. Phase II evaluates safety and efficacy, with inclusion of a primary efficacy endpoint, improvement from Baseline in microperimetry, evaluated at 3 months, and safety and secondary efficacy evaluated at 1-, 6-, 9- and 12 -months post-treatment. All Part II subjects treated under previous versions of the protocol	Updated study design overview for Parts I and II to include expanded description of individual parts

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		will be followed for 12 months with the pre-specified visit schedule. At study completion, all subjects will be invited to participate in a separate long-term follow-up study that will collect efficacy and safety data up to 5 years from surgery.	
	~~The study will~~ consist of visits over a ~~24~~-month evaluation period.	Part I consists of 12 visits over a 24-month evaluation period. Part II consists of 10 visits over a 12-month period	Differentiated Part I and Part II visit number and duration
		To minimise inflammation resulting from surgery and/or vector/transgene, in Part I, all subjects will be given a 21-day course of oral corticosteroid. In Part II, all subjects will be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), placed behind the globe via a deep sub-Tenon approach. Subjects will also be given a 9-week course of oral corticosteroid starting 3 days before surgery: 21 days at 60 mg, followed by 6 weeks of tapering doses. The dose regimen is adjusted for pediatric subjects treated in Part II (see Section 9.8). Subjects in the untreated control group will receive study-visit telephone calls to monitor AEs/SAEs and review concomitant medications on Visit 2 (Day 0) and on Visit 3 and 4 (post-operative Days 1 and 7).	• To improve safety and to prevent potential inflammatory response to therapy, the steroid regimen has been modified. A standardized adult starting dose of 60 mg has been selected (rather than the previous 1mg/kg starting dose) and the taper has been lengthened to include coverage out to approximately 2 months. A 2-month visit has been added to assess the subjects at the end of the steroid therapy. • The treatment of untreated subjects at Visits 2, 3 and 4 has been added
	The efficacy evaluation will be based on BCVA, SD-OCT, fundus autofluorescence,	The efficacy evaluation will be based on microperimetry, BCVA, SD-OCT, fundus autofluorescence, visual fields (Octopus 900 specified	• [redacted]

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~microperimetry,~~ visual fields, contrast sensitivity, low luminance visual acuity (LLVA), ~~full-field stimulus threshold test (FST), colour vision, and reading test.~~ 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 surgery is performed, it should be carried out at least 4 weeks before Visit 1 (Year 1) ~~or Visit 11 (Year 2).~~ A subject is considered to have completed the study if he completes the ~~Year 2~~ assessments. The end of the trial is the date the last subject completes his ~~Year 2~~ 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.	in Part II only), contrast sensitivity, low luminance visual acuity (LLVA), [redacted] and [redacted] Subjects who develop cataracts may undergo cataract surgery if deemed clinically necessary; if surgery is performed, it should be carried out at least 4 weeks before their final visit. A Part I subject is considered to have completed the study if he completes the Month-24 assessments. A Part II subject is considered to have completed the study if he completes the Month 12 assessments. The end of the trial is the date the last subject completes his final-visit 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.	[redacted]; • All above only in Part II • Specified Octopus 900 only in Part II for visual field assessment • Removed assessments that have caused unnecessary study burden in subjects and that have not provided valuable information in Part I • Differentiated duration in Part I and Part II as well as definition of study completion
Section 7.1.3. Dose Expansion Page 25	~~Once the MTD has been identified, u~~Up to 45 additional subjects will be randomized, in a ~~2~~:1 allocation ratio^^ . ~~Subjects will receive AAV8-RPGR either at the MTD (MTD cohort), or at a low dose (active-control cohort), three dose levels below the MTD (e.g., low dose = 5 5 x x 1010 gp if MTD = 5x1011 gp).~~	Up to 45 additional subjects will be randomized in a 1:1:1 allocation ratio to a high-dose group (2.5 × 10^11 gp), a low-dose group (5 × 10^10 gp), and an untreated group. Study data will be collected for both eyes of each subject. Since treatment requires an invasive surgical procedure under general anaesthesia, the sponsor, investigator and the subject will be unmasked to the study procedure (i.e., vitrectomy and sub-retinal injection), however	Part II description now identifies treatments as dose 5 and dose 3 and untreated arm added, per general rationale (see above). Description of assessor masking added

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~Part II of the study will be randomized and double-masked to the assigned dose, and open-label to the treatment administration.~~	within the treated groups, the sponsor, investigator and subject will be masked to the assigned dose. To further minimise potential bias of the treated and non-treated eye evaluations, all subjective ophthalmic assessments at the Screening/Baseline Visit (Visit 1) and from Month 1 (Visit 5) onwards will be conducted by a masked assessor.	
Section 7.3 Discussion of Study Design and Dose Selection Page 25-27	The planned sample size is consistent with a 3+3 escalation scheme. A prospective trial period of 24 months is considered to be a sufficient period of time to monitor for any AEs related to the vector and/or transgene/administration procedure.	The planned sample size in Part I is consistent with a 3+3 escalation scheme. A prospective trial period of 24 months is considered to be a sufficient period of time to monitor for any AEs related to the vector and/or transgene/administration procedure.	Specified Part I for dose escalation
Section 7.3 Discussion of Study Design and Dose Selection Pages 25-27	Further details are provided in the Investigator's Brochure.~~, and a summary of the AAV8-RPGR doses in the toxicology species is presented in the table below. The safety and efficacy findings from other pre-clinical and clinical studies with AAV8 vector for subretinal delivery are also included for comparison.~~	Further details are provided in the Investigator's Brochure.Given previous experience demonstrating the safety of higher subretinal doses of AAV8 vector (Vandenberghe 2011), and lacking safety signals at the lower range of doses, it should be possible to dose-escalate to the high end of the dose range of AAV8-RPGR	This information was removed as it is redundant with the IB. The added language clarified the overall meaning.
	~~Table 1: Toxicology Safety Margin for Clinical Trials~~		Eliminated the table from the protocol as this is redundant with the IB.
	Once ~~the MTD has been identified~~ and the safety and tolerability of AAV8-RPGR is demonstrated in adults, subjects ≥10 year age will be enrolled in Part II of the study. The 10-years of age cut-off safeguards…	Once Part I dose-escalation has been completed, and the safety and tolerability of AAV8-RPGR is demonstrated in adults, subjects ≥10 years of age will be enrolled in Part II of the study. The 10-years of age cut-off safeguards…	Clarified that dose escalation completion is trigger to Part II

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	In Part II, subjects will be randomized to '~~MTD cohort' or the 'active control. The active control cohort will be three dose levels below the MTD. This assures a 1-1.5 log difference in dose between these two cohorts, and allows for identifying a dose response while mitigating the possibility of a subtherapeutic low dose.~~	In Part II, subjects will be randomized to a high-dose (2.5 × 10^11 gp), a low-dose (5 × 10^10 gp), and an untreated group. This allows for comparisons in a randomized and controlled fashion, as recommended by regulators for best practices in ophthalmic gene therapy studies (Human Gene Therapy for Retinal Disorders, Draft Guidance for Industry July 2018). The sponsor, investigator and the subject will be unmasked to the study procedure and treatment (i.e. vitrectomy and sub-retinal injection). However, within the treated groups, the sponsor, investigator and subject will be masked (i.e. double-masked) to the assigned dose. To further minimise potential bias of the treated and non-treated eye evaluations, all subjective ophthalmic assessments at the Screening/Baseline Visit (Visit 1) and from Month 1 (Visit 5) onwards will be conducted by a masked assessor.	Part II description now identifies treatments as dose 5 and dose 3 and untreated arm added, per general rationale (see above). Added description of masking procedures
Section 8.0 Selection and Withdrawal of Subjects Page 28		8.1 Part I	Differentiating Part I and Part II inclusion criteria to improve clarity
Section 8.1 Part I Section 8.1.1 Inclusion Criteria Pages 28	Subject~~/ parent (if applicable)~~ is willing and able to give informed consent for participation in the study		• Part I is only in adults so removed the language for minors
Section 8.1 Part I Section 8.1.1 Inclusion Criteria Page 28	3. Are male and able to comply and adequately perform all study assessments ~~Part I:≥18 years of age~~ ~~Part II: ≥10 years of age~~	Part I Are male, ≥18 years of age, and able to comply and adequately perform all study assessments.	Clarified age inclusion for Part I; • separated Part I and Part II inclusion to add clarity for investigators;
Section 8.1 Part I Section 8.1.1 Inclusion Criteria Page 28	5. BCVA in both eyes that meets the following criteria, based on the cohort level:	5. BCVA in both eyes that meets the following criteria, based on the cohort level: Cohort 4-6: better than or equal to BCVA of 34 ETDRS letters	• Removed Part II because inclusion criterion is now separated for Part

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	Cohort 4-6 ~~and study Part II~~: better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity)	(equivalent to better than or equal to 6/60 or 20/200 Snellen acuity)	II to improve clarity
Section 8.1.1 Selection and Inclusion Criteria Page 28	*~~Prior d~~ocumentation…*	*D*ocumentation	• *Clarified documentation requirement*
Section 8.2 Part II Section 8.2.1 Inclusion Criteria Page 28		Part II	• Differentiating Part I and Part II inclusion criteria to improve clarity
Section 8.2 Part II Section 8.2.1 Inclusion Criteria Pages 28-29		Part II Inclusion Criteria 1. Subject / parent (if applicable) is willing and able to provide informed consent for participation in the study 2. Are male, ≥10 years of age, and able to comply and adequately perform all study assessments 3. Documentation of a non-synonymous mutation in the RPGR gene 4. Have active disease clinically visible within the macular region in the study eye and defined as follows: ➢ ellipsoid zone (EZ) on SD-OCT at screening must be measurable, and within the nasal and temporal border of any B-scan, and not be visible on the most inferior and superior B-scan 5.Have a BCVA in the study eye that meets the following criteria: •Better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity). 6. Mean total retinal sensitivity in the study eye as assessed by microperimetry ≥0.1 dB and ≤8 dB	• Added section for Part II inclusion criteria to clarify the differences between Parts I and II; • EZ zone must be measurable on screening in the study eye • BCVA is same for all subjects in Part II (no cohort differences); specifies criterion is for study eye only • Added mean total retinal sensitivity range in the study eye to ensure that subjects have modifiable microperimetry not subject to ceiling effects

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 8.3. Exclusion Criteria: Parts I and II Page 29		Parts I and II	To clarify that exclusion criteria are the same for Parts I and II
Section 9.1 Treatments Administered Page 31	~~At the Injection Day Visit (Visit 2, Day 0),~~ subjects will undergo vitrectomy and retinal detachment in their study eye and then receive a single, sub-retinal injection of AAV8-RPGR (See Section 9.4 for details).	Part I, Dose Escalation, Phase 1: all subjects will undergo vitrectomy and retinal detachment in their study eye and then receive a single, sub-retinal injection of AAV8-RPGR (See Section 9.4 for details). Part II, Dose Expansion, Phase 2/3: Subjects will be assigned to 1 of the following: high-dose (2.5 × 10^11 gp), low-dose (5 × 10^10 gp), or an untreated control arm.	Clarified the treatments in Part I and Part II
Section 9.3. Packaging, Labeling, Preparation and Storage Page 31	The Investigational Medicinal Product will be labelled in compliance with regulatory standards. ~~(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, (IMP labellied for Europe only) storage conditions and caution statement.~~	The Investigational Medicinal Product (IMP) will be labelled in compliance with regulatory standards.	Deleted label information.
Section 9.4 Vitrectomy Procedure and Injection of AAV8-RPGR Pages 31		The subretinal injection technique to be used in this study is similar to that developed in the sponsor's choroideremia programme in Oxford… To date, over 150 subjects have been injected without complication by retinal surgeons using the technique described below. All subjects will be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), placed behind the globe via a deep sub-Tenon approach.	Updated vitrectomy procedure background and added description of additional local steroid administration at the time of surgery
Section 9.5 Randomisation Page 33	~~The~~ dose-escalation portion of this study is not randomized. In Part II, after the study eye is assigned, subjects will be randomised ~~a 2:1 ratioAAV8-RPGR MTD~~	Part I, the dose-escalation portion of this study, is not randomized. In Part II, after the study eye is assigned, subjects will be randomised to 1 of 3 groups with a 1:1:1 allocation ratio: 1) treatment with AAV8-RPGR at a high dose (2.5 × 10^11 gp); 2) treatment with	Part II randomization procedure added; doses identified as dose 5 and dose 3 and untreated arm added, per

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~or a lower dose of AAV8-RPGR, three dose-levels from MTD (e.g., low dose = 5 x 10^{10} gp if MTD = 5x10^{11} gp) for the active control cohort.~~	AAV8-RPGR at a low dose (5 × 10^10 gp) or 3) no treatment.	introductory general rationale.
Section 9.6. Study Masking Page 33-34	Part II is ~~double~~ masked (subject, surgeon, investigator/site team, sponsor will be masked to the assigned dose, and open-label with respect to the treatment administration).	In Part II, all ophthalmic assessments that are conducted at the Screening/Baseline Visit will be conducted by appropriately qualified masked assessors. For the immediate post-operative visits, masking of the assessors will not be viable as clinical signs of surgery will be apparent (i.e. redness, swelling). Therefore, unmasked assessors will perform all ophthalmic assessments at Visit 3 (Day 1) and Visit 4 (Day 7). For Visit 5 (Month 1) onwards, masked assessors will be used, as any signs of surgery will have dissipated and it will not be possible clinically to differentiate between those subjects that have not undergone surgery, and those subjects that have undergone surgery and received active treatment. Subjects randomised to the untreated Control group will not be required to attend the site at Visit 2, 3 or 4. As the key purpose of Visit 2 is surgery, and Visit 3 and 4, post-operative safety, there is limited utility in Control subjects attending. Therefore, to limit the study burden for Control subjects thereby potentially reducing the risk of subject withdrawal at this stage and reducing the possibility of further unmasking due to direct contact and communication with fellow participants, Control subjects are not scheduled to attend the clinic for study visits at these times. In order to minimise bias further, masked assessors will not have access to the subject's medical records, source documentation or eCRF as data entries or notation	Clarified masking procedure

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		(such as use of peri-operative corticosteroid) may be sources of unmasking. From Visit 5 (Month 1) onwards, the masked assessor will also read a pre-written statement to each subject, regardless of randomisation, reminding them of the masked nature of the study, and to avoid any reference to prior surgery/non-surgery, which eye may have received treatment or to allude to any information that may unmask the assessor as to which group the subject has been assigned to. Furthermore, it is anticipated that a subset of the subjects participating in the trial will be active on social media. Following appropriate approval by the Independent Ethics Committee (IEC)/Institutional Review Board (IRB), the patient information leaflet will request that subjects refrain from posting any details of study participation on social media, that may unmask the assessors to the group the subject has been assigned to. This request will be reiterated at subject visits by the investigator and within the pre-written statement. Subjects randomised to the AAV8-RPGR treatment groups, surgeons, the investigative team and the study sponsor will be masked to which dose of AAV8-RPGR the subject has been assigned to. Unmasked study site personnel will be assigned the responsibility of performing dilution, which will take place in a designated area remote from the investigative team to preserve masking of the treatment arm. Personnel delegated to perform the dilution will not be involved in any other aspect of the study (i.e., consent, safety/efficacy assessments, surgical procedure).	
Section 9.8 Concomitant Therapy		To minimise inflammation, at the time of surgery, all subjects will be	• To improve safety and to

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Pages 34-35		treated with a course of corticosteroid. In Part I, all subjects will also be prescribed a 21-day course of oral prednisone/prednisolone, following closely the 17-day protocol established in the Philadelphia AAV gene therapy clinical trial (Maguire et al., 2008), except allowing an extra 4 days for tapering the dose at the end of the course, i.e., 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. In Part II, at the time of surgery, all subjects (adult and pediatric) will be treated with up to 1 mL of triamcinolone, 40 mg/mL solution, which must be placed behind the globe via a deep sub-Tenon approach. In addition, all subjects will be prescribed a course of oral corticosteroids. For adults, 60 mg of oral prednisone / prednisolone will be prescribed for the initial 21 days (starting 3 days prior to surgery), followed by a weekly taper as follows for a total of 9 weeks of treatment: Day -3 through day 17 (21 days): 60 mg by mouth once daily Day 18 through day 24 (7 days): 50 mg by mouth once daily Day 25 through day 31 (7 days): 40 mg by mouth once daily Day 32 through day 38 (7 days): 40 mg by mouth once daily Day 39 through day 45 (7 days): 20 mg by mouth once daily Day 46 through day 52 (7 days): 10 mg by mouth once daily	prevent potential inflammatory response to therapy, the steroid regimen has been modified. A standardized adult starting dose of 60 mg has been selected (rather than the previous 1mg/kg starting dose) and the taper has been lengthened to include coverage out to approximately 2 months. A 2-month visit has been added to assess the subjects at the end of the steroid therapy. • A pediatric regimen has also been added • General safety information regarding the use of steroids has been added

		Day 53 through day 59 (7 days): 5 mg by mouth once daily. If at the Month-2 visit (Visit 5.9), inflammation is observed, corticosteroid therapy should be re-initiated, via oral and/or intraocular route, based on the clinical condition of the subject, and the judgement of the investigator. For pediatric subjects, oral prednisolone/prednisone will also be started 3 days prior to surgery. The starting dose will be based on kilogram weight of the subject, up to a maximum of 60 mg starting dose (rounded to the nearest 1 mg). Subsequent doses will have multipliers to provide the appropriate taper over an additional 6 weeks, for a total of 9 weeks of treatment. See tapering regimen for pediatric subjects below: Day -3 through day 17 (21 days): Starting Dose (SD) 1 mg/kg by mouth once daily (maximum dose of 60 mg/once daily) Day 18 through day 24 (7 days): SD X 0.83 mg by mouth once daily Day 25 through day 31 (7 days): SD X 0.67 mg by mouth once daily Day 32 through day 38 (7 days): SD X 0.5 mg by mouth once daily Day 39 through day 45 (7 days): SD X 0.33 mg by mouth once daily Day 46 through day 52 (7 days): SD X 0.17 mg by mouth once daily Day 53 through day 59 (7 days): SD X 0.08 mg by mouth once daily If at the Month-2 visit (Visit 5.9), inflammation is observed, corticosteroid therapy should be reinitiated, via oral and/or intraocular route, based on the clinical condition of the subject, and the judgement of the investigator. The local pediatric team should be involved with all children undergoing gene therapy surgery and should be available to give advice on the steroid doses used in	

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		each patient. Modification of the protocol-defined steroid treatment are allowed based on recommendations of the pediatric team with approval from the Sponsor. For all subjects, while taking oral steroids, special note should be made to follow for potential side effects (e.g., increased IOP, cataracts, hypertension, elevated blood sugar, infections, gastritis/peptic ulcer disease, edema, electrolyte imbalance, mood changes, insomnia), and appropriate prophylaxis and/or therapy should be instituted as needed (e.g., treatment with proton pump inhibitors and/or valcyclovir; restriction of nonsteroidal anti-inflammatory agents)	
Section 10.1 Visit 1 (Screening/Baseline Visit) Pages 37-38		The subject or parent will sign and date one copy of the consent form in the presence of the investigator or his/her designee; where applicable, an assent form will be completed by the subject.	• Moved for clarity
Section 10.1 Visit 1 (Screening/Baseline Visit) Pages 38	• For Part I, RPGR gene mutation screen (only if not conducted previously); for Part II, ~~prior~~ documentation of a non-synonymous mutation in the RPGR gene •~~Colour vision test~~ •~~Speed reading test~~ •~~FST~~ Footnote: ~~It is recommended to measure BCVA and LLVA twice on the first day and once on the second day (prior to pupil dilation). All 3 BCVA and all 3 LLVA values must be recorded in the eCRF. The highest BCVA score will be used to~~	• For Part I, RPGR gene mutation screen (only if not conducted previously); for Part II, documentation of a non-synonymous mutation in the RPGR gene • ETDRS BCVA[1] [redacted] • Fundus autofluorescence • MAIA Microperimetry[4] • Fundus photography • Octopus 900 visual fields[4,5] • Contrast sensitivity test • AE and SAE monitoring [redacted] • Randomisation[3] **Footnotes:** 1. For Part I subjects, BCVA is performed in triplicate at baseline. For Part II subjects, if the BCVA	• Includes specifics on genetic diagnosis criterion • Assessments at Visit 1 now include AO-OCT, [redacted] • Specifies Octopus 900 visual field • AE and SAE monitoring will occur from screening through end of study instead of SAE monitoring only

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~define subject eligibility. LLVA should be conducted immediately after each BCVA assessment.~~	value at Visit 1 (Screening/Baseline) is ≥ ± 10 letter gain or loss in the study eye compared to the previous XOLARIS study visit (if applicable), then BCVA must be repeated an additional 2 times, resulting in a total of 3 BCVA measures at Visit 1. For Part II subjects, if the BCVA value at Visit 1 (Screening/Baseline) is < ± 10 letter difference in the study eye compared to the previous XOLARIS study visit, then BCVA will be collected once and will not be repeated. If subject was not previously in XOLARIS study, BCVA assessments at baseline must be triplicate. For all subjects who require triplicate BCVA testing, to facilitate the additional BCVA measures, this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF. The highest score will be used to define subject eligibility. [redacted] 3. Part II only. 4. Assessments collected in triplicate. To facilitate triplicate testing, the visit should be conducted over 2 days. Visual field and microperimetry outputs will be sent to a CRC for review. Data will be generated and collated within the CRC and exported to the Sponsor or designee for inclusion in the study database.	• Removed reading test, colour vision and FST • Removed footnote information regarding BCVA and LLVA; added information in footnote related to Octopus 900 and new assessments • Added new information regarding baseline BCVA assessment requirements • [redacted] • Specified in footnote that Octopus 900 is only specified in Part II • Moved text for BCVA timeline

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		5. Octopus 900 perimetry is specified for visual field assessments only in Part II. [redacted]	
Section 10.1 Visit 1 (Screening/Baseline Visit) Page 38	In Part II, subjects will be then randomised to one of the AAV8-RPGR treatment groups ~~(MTD cohort or active control cohort) and subjects receiving treatment will remain masked to the treatment dose.~~	In Part II, subjects will be informed of the randomisation outcome (i.e., AAV8-RPGR treatment or the Control group) and instructed to not reveal their treatment group assignment to the masked assessors during the study. Subjects randomised to the AAV8-RPGR treatment groups (along with the Investigators and sponsor) will remain masked to the assigned dose.	New randomization and treatments specified
Section 10.1 Visit 1 (Screening/Baseline Visit) Page 38	Subjects will be given a ~~21-day~~ course of oral prednisone/prednisolone	Subjects will be given a 21-day course of oral prednisone/prednisolone and instructed to start taking the drug 2 (Part I) or 3 (Part II) days before their next study visit (Visit 2).	Steroid course is no longer 21 days for all subjects
Section 10.1 Visit 1 (Screening/Baseline Visit) Page 38 Footnote 1		For Part II subjects, if…	Clarified for Part II subjects
		For all subjects who require triplicate BCVA testing, to facilitate the additional BCVA measures, this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF. The highest score will be used to define subject eligibility.	Added text to specify subjects and added information moved from previous location
		, and	Clarified grammar
Section 10.2 Visit 2 (Day 0, Surgery/Injection Day Visit) Pages 38-39		At Visit 2, all subjects in the AAV8-RPGR groups will visit the surgical site, and the following assessments will be performed prior to surgery: • Full ophthalmic examination, including indirect ophthalmoscopy, slit lamp examination with IOP assessment, anterior chamber and vitreous inflammation grading, and LOCS III cataract grading	• Ophthalmic exam has been added • Language added because one arm is untreated in Part II • Added language specifying that other non-study procedures may be necessary but

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		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 analyses. All subjects will be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), placed behind the globe via a deep sub-Tenon approach. Subjects in the Control group will receive a study visit Telephone Call at the agreed upon date and time. Sites will conduct the following assessments during the telephone call: • AE/SAE monitoring • Concomitant medication review.	will not be collected • Added new local steroid therapy added at time of surgery • Added how the untreated arm will be assessed through a telephone call at that visit.
Section 10.2 Visit 2 (Day 0/ Surgery/Injection Day Visit) Page 38-39 Footnote		• *Corticosteroid compliance review*[1] *1. Corticosteroid review is applicable only for treated subjects.*	• *Added footnote that specifies corticosteroid review is only for treated subjects.*
Section 10.3 Visit 3 (Day 1 Post-Operative Visit) Page 39		Subjects in the Control group will receive a study visit Telephone Call at the agreed upon date and time. Sites will conduct the following assessments during the telephone call: • AE/SAE monitoring • Concomitant medication review.	Added how the untreated arm will be assessed through a telephone call at that visit.
Section 10.3 Visit 3 (Day 1 Post-operative Visit) Page 39 Footnote		• *Corticosteroid compliance review*[1] *1. Corticosteroid review is applicable only for treated subjects.*	*Added footnote that specifies corticosteroid review is only for treated subjects.*
Section 10.4 Visit 4 (Day 7 Post-Operative Visit) Page 40		Subjects in the Control group will receive a study visit Telephone Call at the agreed upon date and time. Sites will conduct the following	Added how the untreated arm will be assessed through a

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		assessments during the telephone call: • AE/SAE monitoring • Concomitant medication review.	telephone call at that visit.
Section 10.4 Visit 4 (Day 7 Post-operative Visit) Page 40 Footnote		• *Corticosteroid compliance review*[1] *1. Corticosteroid review is applicable only for treated subjects.*	*Added footnote that specifies corticosteroid review is only for treated subjects.*
Section 10.5 Visit 5 (Month 1) Page 40-41		• MAIA Microperimetry	• Specified MAIA microperimetry
Section 10.5 Visit 5 (Month 1 Visit) Page 41 Footnote		• *Corticosteroid compliance review*[1] • *1. Corticosteroid review is applicable only for treated subjects.*	• *Added footnote that specifies corticosteroid review is only for treated subjects.*
Section 10.6 Visit 5.9 (Month 2) Page 41		At Visit 5.9, the following assessments will be performed: • Collection of safety blood samples (haematology and clinical chemistry) • Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading • ETDRS BCVA • SD-OCT • Fundus autofluorescence • MAIA Microperimetry • Viral shedding • Immunogenicity sampling • AE/SAE monitoring • Concomitant medication review • Corticosteroid compliance review	New visit added to better monitor safety of subjects This visit is denoted as Visit 5.9 in order to maintain the visit numbering in the EDC
Section 10.6 Visit 6 (Month 2 Visit) Page 41 Footnote		• *Corticosteroid compliance review*[1] • *1. Corticosteroid review is applicable only for treated subjects.*	• *Added footnote that specifies corticosteroid review is only for treated subjects.*

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 10.7 Visit 6 (Month 3) Page 41-42	• ~~Colour vision test~~ Footnote: ~~It is recommended to measure BCVA and LLVA twice on the first day and once on the second day (prior to pupil dilation). All 3 BCVA and all 3 LLVA values must be recorded in the eCRF. LLVA should be conducted immediately after each BCVA assessment.~~ • ~~*Fundus photography*~~ • ~~*Viral shedding*~~	• MAIA Microperimetry • Octopus 900 visual fields[1] [redacted] Footnotes: 1. Octopus 900 perimetry is specified for visual field assessments only in Part II. [redacted]	• Month 3 added new assessments; • removed color vision test • specified MAIA microperimetry [redacted] • Specified in footnote that Octopus 900 is only specified in Part II • Removed footnote information in BCVA and LLVA • *Removed assessments not done at visit*
Section 10.8. Visit 7 (Month 6) Pages 42	• ~~Colour vision test~~ • ~~Speed reading test~~ • ~~FST~~ • ~~*Fundus photography*~~	[redacted] • MAIA Microperimetry • Octopus 900 visual fields[2] [redacted] Footnotes: [redacted] 2. Octopus 900 perimetry is specified for visual field assessments only in Part II.	• Removed color vision, speed reading, *fundus photography,* and FST; [redacted] • specifies MAIA microperimetry and Octopus 900 visual field
Section 10.9. Visit 8 (Month 9) Page 42-43	• ~~Fundus autofluorescence~~	• MAIA Microperimetry	• Removed autofluorescence; • MAIA microperimetry specified

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 10.10. Visit 9 (Year 1) Pages 43	• ~~Colour vision test~~ • ~~Speed reading test~~ • ~~FST~~	• BCVA[1] [redacted] • MAIA Microperimetry • Octopus 900 visual fields[3] [redacted] Footnote: *1. (see below)* [redacted] 3. Octopus 900 perimetry is specified for visual field assessments only in Part II. [redacted] Subjects who develop cataracts may undergo cataract surgery if deemed clinically necessary; if surgery is performed, it should be carried out at least 4 weeks before Visit 9 (Year 1) or Visit 11 (Year 2) for Part II and Part I subjects, respectively.	• Removed color vision, reading and FST • Added [redacted] • Specified MAIA microperimetry and Octopus 900 visual field with associated footnotes • Differing times for Part I and Part II for window between potential cataract surgery and end of study visits [redacted] • Specified in footnote that Octopus 900 is only specified in Part II
		ETDRS BCVA[1]	*Clarified footnote for BCVA assessments in 7.1*
	~~*OCT*[1]~~	*OCT*[2]	*Updated footnote*
	~~*fields*[2]~~ [redacted]	*fields*[3] [redacted]	*Updated footnote* [redacted]
		1. Part I subjects perform BCVA assessments in triplicate at Year 1. To facilitate the additional BCVA measures, this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation).	*Clarified footnote for BCVA assessments for Part I subjects only in 7.1*

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		All 3 BCVA values must be recorded in the eCRF.	
	~~*2, 3*~~	*2., 3, 4*	*Updated footnote numbers to accommodate new footnote 1 added.*
Section 10.11. Visit 10 (Month 18) Page 44		(Part I Subjects Only)	Specified that Visit 11, Month 18 is only for subjects of Part I
		MAIA	Added to Microperimetry
Section 10.12. Visit 11 (Year 2) Page 44	• ~~Colour vision test~~ • ~~Speed reading test~~ • ~~FST~~	(Part I Subjects Only)	• Specified that Visit 12, Month 24 is only for subjects of Part I • Removed assessments
		• *MAIA*	• *Added to Microperimetry*
		• *ETDRS BCVA*[1] *1. Part I subjects perform BCVA assessments in triplicate at Year 2. To facilitate the additional BCVA measures, this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF.*	• *Specified that BCVA assessments are performed in triplicate at years 2 and visit should be conducted over 2 days.*
Section 10.13 Early Termination (ET) Visit Page 45	• ~~Colour vision test~~ • ~~Speed reading test~~ • ~~FST~~	[redacted] • MAIA Microperimetry • Octopus 900 visual fields[2] [redacted] Footnotes: [redacted] 2. Octopus 900 perimetry is specified for visual field assessments only in Part II. 3. Immunogenicity sampling at the ET Visit is to be conducted only if visit occurs prior to Year 1 visit. 4. Part II only.	• Added to ET visit, if possible, [redacted], MAIA Microperimetry, [redacted], and associated footnotes • Removed colour vision test, reading test, FST [redacted]
Section 10.13		• *Immunogenicity sampling*[3]	• *Added footnote*

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Early Termination (ET) Visit Page 45		• *25[4]*	• *Updated footnote*
		• *Immunogenicity sampling at the ET Visit is to be conducted only if visit occurs prior to Year 1 visit.*	• *Footnote added to clarify assessment requirements*
		• *4., and*	• *Updated footnote number, clarified grammar*
Section 10.14 Unscheduled Visits Page 45-46		• *Autofluorescence*	• *Added assessment to visit*
		• *MAIA*	• *Added to Microperimetry*
		• Microperimetry, *if clinically feasible*	• Added microperimetry to unscheduled visit, *if clinically feasible*
Section 11.1 Best Corrected Visual Acuity Page 47	BCVA will be performed in triplicate over a 2-day period at Visits 1,9-and 11 (or ET Visit) for all subjects. It is recommended that BCVA will be conducted twice on the first day and once on the second day. All values will be entered in the eCRF.	For Part I subjects, BCVA will be performed in triplicate over a 2-day period at Visits 1, 9 and 11 (or ET Visit) for all subjects. It is recommended that BCVA will be conducted twice on the first day and once on the second day. All values will be entered in the eCRF. For Part II subjects, if the BCVA value at Visit 1 (Screening/Baseline) is ≥ ± 10 letter gain or loss in the study eye compared to the previous XOLARIS study visit (if applicable), then BCVA must be repeated an additional 2 times, resulting in a total of 3 BCVA measures at Visit 1. To facilitate the additional BCVA measures this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF. The highest score will be used to determine subject eligibility. If the BCVA value at Visit 1 (Screening/Baseline) is < ± 10 letter difference in the study eye	New instructions on BCVA triplicate requirement

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		compared to the previous XOLARIS study visit, then BCVA will be collected once and will not be repeated. If subject was not previously in XOLARIS study, BCVA assessments at baseline must be performed in triplicate.	
[redacted]		[redacted]	[redacted]
Section 11.5 MAIA Microperimetry Page 48		MAIA Microperimetry will be conducted for both eyes at the times indicated in Table 3, Section 17.1. Microperimetry will be performed on both eyes in triplicate over a 2-day period at Visit 1 for all subjects. The final assessment should be used to determine subject eligibility. If at subsequent visits there are obvious technical challenges or the subject is not performing the assessment as expected from previous visits (e.g. distracted, large number of false positive responses, not maintaining fixation, etc), then the assessment may be repeated and the second assessment should be used for that study visit.	Specifies that MAIA microperimetry will be performed in triplicate at Visit 1 only or if there is an inconsistent reading at other visits
Section 11.6 Visual Fields Page 48		In Part II, visual fields should be assessed using the Octopus 900 perimeter.	Specifies that Octopus 900 visual field will be conducted during Part II.
Section 11.7. Contrast Sensitivity Page 49	~~Contrast sensitivity will be measured prior to pupil dilation using a Pelli Robson chart.~~		No longer specifies using the Peli-Robson chart.

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 11.8 Low Luminance Visual Acuity Page 49	The test should be performed ~~after~~ before BCVA testing and ~~prior to~~ pupil dilation. ~~LLVA should be performed in triplicate over a 2-day period at Visit 1 and , Visit 9 8 (Month 3) and Visit 11 (1 Year) (or the ET Visit) for all subjects, if applicable. It is recommended that LLVA will be conducted twice on the first day and once on the second day. All values will be entered into the eCRF.~~	The test should be performed before BCVA testing and pupil dilation.	LLVA is no longer conducted in triplicate; it is now conducted prior to BCVA testing instead of after
Full Field Stimulus Threshold Test Page 52	~~FST will be measured for both eyes after a period of dark adaptation and at the times indicated in Table 3, Section 17.1 (Visit 1, 7, 9, 11, ET) only at sites where the required FST equipment is available, as specified in the Study Operations Manual. FST measurements will be taken by appropriately qualified technicians. For complete technical specifications, refer to the Study Operations Manual.~~		Removed assessment
Colour Vision Page 52	~~Colour vision will be tested for both eyes prior to pupil dilation, at the times indicated in Table 3, Section 17.1. Eyes will be tested separately and in the same order at each assessment. For colour vision testing, assessors will be appropriately qualified for conducting the assessment.~~		Removed assessment

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Reading Test Page 52	~~Reading performance will be evaluated prior to pupil dilation for both eyes at the times indicated in Table 3, Section 17.1. The reading test will be provided to each site by the Sponsor; for complete user instructions, refer to the Study Operations Manual.~~ ~~For the reading test, assessors will be appropriately qualified for conducting the assessment.~~		Removed assessment
[redacted]		[redacted]	[redacted]
[redacted]		[redacted]	[redacted]

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		only in countries in which it is validated.	
Section 12.2.2 Recording of Adverse Events Page 51		AEs/SAEs… AEs/SAEs that the investigator becomes aware of, and which are deemed to have a relationship to the study drug should continue to be reported to NightstaRx following the completion of the study for a period of up to 5 years following surgery, until/unless the subject is enrolled in another NightstaRx study.	AEs and SAEs will be collected from the time of informed consent to the end of study. New sentence added to mandate collection of AE information from subjects for up to 5 years from the end of study
Section 12.2.4 Reporting of Serious Adverse Events and DLTs Page 51-52	All cases that are fatal or life-threatening will be reported ~~no later than 7 days~~ after the sponsor received the initial report from the Investigator.	The sponsor may unmask any SAE reports that are serious, unexpected, and related to the study drug, as required, in accordance with safety reporting guidance and regulations. All cases that are fatal or life-threatening will be reported immediately after the sponsor receives the initial report from the Investigator.	Added language regarding unmasking per GCPs Shortened window for reporting SAEs per regional regulators
Section 12.2.5 Procedures for Unmasking Page 53	***Procedures for Unmasking*** ***The Investigator has the ability to unmask an individual subject's treatment assignment, ~~but this capability is restricted to medical emergencies in which knowledge of the subject's treatment is critical to the Investigator's ability to treat the subject.~~*** ***~~In such a case, the Investigator must first attempt to contact the Medical Monitor or backup to discuss the rationale for unmasking.~~*** ***If the Investigator determines that ~~the conditions above are met,~~ then s/he unmasks the***	***Procedures for Unmasking*** ***The Investigator has the ability to unmask an individual subject's treatment assignment, in order to provide emergency treatment. Unmasking is appropriate when knowledge of the subject's dose would affect the medical management of the subject.*** ***If the Investigator determines unmasking is required, then s/he unmasks the subject through the Electronic Data Capture (EDC) system, files the resulting confirmation in a restricted file until the end of the study, and notifies the Medical Monitor within 24 hours that the subject's treatment assignment has been unmasked. The Investigator will not communicate the treatment assignment to the Medical Monitor or to any sponsor or study team***	***New section added per GCPs regarding unmasking procedures*** ***Changes to the unmasking procedure have been added to align more closely to GCP guidelines in Version 7.1 to Version 8.0.***

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	subject through the Electronic Data Capture (EDC) system, files the resulting confirmation in a restricted file until the end of the study, and notifies the Medical Monitor within 24 hours that the subject's treatment assignment has been unmasked. The Investigator will not communicate the treatment assignment to the Medical Monitor or to any sponsor or study team staff; nor should the treatment assignment be communicated to site staff other than those personnel who require that knowledge in order to treat the subject. The Investigator will ensure that the rationale, date, and time of unmasking is noted in the subject's source documentation, but not the treatment assignment. The medical emergency that necessitated unmasking must also be recorded in the source documentation and the case report form per standard study procedures.	***staff; nor should the treatment assignment be communicated to site staff other than those personnel who require that knowledge in order to treat the subject. The Investigator will ensure that the rationale, date, and time of unmasking is noted in the subject's source documentation, but not the treatment assignment. The medical emergency that necessitated unmasking must also be recorded in the source documentation and the case report form per standard study procedures.***	
Section 13.1 Sample Size Page 56	A sample size of ~~30~~ subjects ~~at the MTD dose~~ ensures ~~that events with an incidence ≥10% will be identified with a 95% probability.~~	Due to the nature of the study design of Part I, no formal sample size computation was performed. In Part II, A a sample size of 45 subjects, 15 in each of 3 groups (high-dose, low-dose and untreated), ensures 80% power at 0.05 significance level assuming that the treated arm has 50% probability of achieving ≥7 dB improvement at ≥5 loci at Month 3 vs. 5% in the untreated control arm.	Specified Part I and Part II sample size rationale

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 13.3.1 Safety Analysis Set Page 56		The Safety Analysis Set will consist of all subjects who receive study treatment (vitrectomy/AAV8-RPGR) in Part I, and all subjects that are randomized in Part II.	
Section 13.3.2 Full Analysis Set Page 56	The Full Analysis Set will include all subjects for whom data of at least 1 post baseline efficacy assessment is available in ~~at least one~~ eye.	The Full Analysis Set will include all subjects for whom data of at least 1 post baseline efficacy assessment is available in the study eye.	
Section 13.4 Descriptive Statistics Page 56		No formal statistical comparison will be performed in Part I, and statistical tests will be conducted for the efficacy endpoints in Part II.	
	Summaries will be generated by dose and overall, in Part I and, by group (~~MTD~~ dose, ~~and~~ low-dose) in Part II.	Summaries will be generated by dose and overall, in Part I and, by group (high-dose, low-dose, untreated) in Part II.	Included untreated arm
Section 13.5 Demographics and Baseline Characteristics Page 57		Demographics and baseline ocular characteristics will be summarised for the safety analysis set and the full analysis set for Part I and Part II separately.	
Section 13.6 Efficacy Analyses Page 57	Efficacy assessments are ocular in nature and therefore will be tabulated by eye (Study Eye and Fellow Eye). Efficacy data will be summarised using descriptive statistics (described in Section 13.4). ~~Change~~ from baseline in ~~BCVA~~ will be tabulated by visit and by eye~~.~~	Efficacy assessments are ocular in nature and therefore will be tabulated by eye (Study Eye and Fellow Eye). Efficacy data will be summarised using descriptive statistics (described in Section 13.4). This will be done for each cohort in Part I, and each arm in Part II. Improvement in retinal sensitivity and change from baseline in retinal sensitivity will be tabulated by visit and by eye in each Part, and be compared between the randomized groups in Part II by visit for the study eye.	
Section 13.6.1 Multiplicity Adjustment Page 57	~~Alpha adjustment is not applicable in this exploratory Phase 1/2 study~~ study.	No multiplicity adjustment is planned in the study. Part I is the dose-escalation to identify the MTD based on the evaluation of benefit and risk without any formal statistical comparisons, and therefore no multiplicity adjustment is needed. In Part II, the analysis at 3 months for the primary efficacy	Specifies Part I and Part II multiplicity adjustment requirements

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		endpoint will determine whether the study is positive or not. Each dose will be compared with the untreated control separately by Fisher's Exact Test at 0.05 level (two-sided).	
Section 13.8 Interim Analyses Page 58	~~In Part II, secondary endpoints will be analysed at 3, 6, 12, 18 and 24 months with masking to treatment dose maintained.~~	In Part II, the analysis after all subjects complete 3-month visit will evaluate the primary efficacy endpoint, and hence is not an interim analysis. The personnel who are involved in daily operation will continued to be masked of the treatment information until study completion to minimize any operational bias. Additional analysis and a final analysis will be conducted for regulatory interactions.	
Section 14.1 Informed Consent Page 59		This is an assessor-masked study. All subjects will be informed at the time of randomisation whether they have been randomised to the AAV8-RPGR or Control group. However, subjects in the AAV8-RPGR treatment groups will be masked to the assigned dose.	Masking and randomization information have been added
Section 15.2 Data Handling and Records Management Page 61	~~(by KCT Data).~~ ~~(by KCT Data).~~		Removes specification for KCT Data
Section 15.4 Time and Schedule of the Study Page 62	~~The expected overall study duration is 36 months.~~	Part I includes 24 months of follow-up for each subject enrolled and treated. Part II includes 12 months of follow-up for each subject enrolled and treated.	Duration changed to 24 months for overall study duration of Part 1. This has been corrected to define the actual duration per subject and not the estimated duration to last patient last visit. Part II is defined similarly, i.e., with 12 months of follow-up for each subject.

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 17.1 Schedule of Study Procedures Page 68-71			• Changes reflect changes in assessments detailed above under individual visits • Abbreviations updated • Footnotes updated
Section 17.1 Schedule of Study Procedures Page 68-71	a. ~~Subjects will take 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).~~	Footnotes	Removed details on steroid regimen. This information can be found in body of protocol.
Section 17.1 Schedule of Study Procedures Table 2, Pages 68-71			
Visit Number		Visit 2[e], Visit 3[e], Visit 4[e]	Added footnote to Visits 2-4 to clarify telephone call procedure
Safety blood samples	~~Samples~~[e]	Samples[f]	Updated footnote reference
RPGR mutation screen	~~screen~~[f]	Screen[g]	Updated footnote reference
Full ophthalmic examination	~~examination~~[g]	examination[h]	Updated footnote reference
Surgical procedure/dosing	~~Dosing~~[h]	Dosing[i]	Updated footnote reference
Dispensation of oral steroids	~~Dispensation of oral steroids~~[i]	Dispensation of oral steroids[j]	Updated footnote reference
EDTRS BCVA	~~BCVA~~[j]	BCVA[k]	Updated footnote reference

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
[redacted]	[redacted]	[redacted]	[redacted]
LLVA	~~X~~		Deleted LLVA procedure at Visit 5.9, procedure not done.
Fundus autofluorescence		X	Added Fundus autofluorescence to Visit 5 in table
MAIA Microperimetry	~~Microperimetry~~[l]	Microperimetry[m]	Updated footnote reference
Fundus photography	~~X~~		Deleted Fundus photography from Visit 7, procedure not done
Visual fields	Visual fields (Octopus 900 in Part II)[m]	Visual fields (Octopus 900 in Part II)[n]	Updated footnote reference
	~~X~~	X	Added Visual Fields to Visit 6, deleted Visual Fields from Visit 10
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]
Viral shedding	~~shedding~~[n]	shedding[o]	Updated footnote reference
Contrast sensitivity	~~X~~		Removed procedure from Visit 10
Immunogenicity sampling	~~sampling~~[o]	sampling[p]	Updated footnote reference

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~X~~[n]	X[p]	Updated footnote reference
AE, SAE monitoring	~~monitoring~~[p]	monitoring[q]	Updated footnote reference
Corticosteriod compliance review		review[r]	Added footnote to clarify corticosteroid procedure
Randomisation	Randomisation[q]	Randomisation[s]	Updated footnote reference
[redacted]	[redacted]	[redacted]	[redacted]
Footnote d		if clinically feasible	Added to clarify assessment procedure
Footnote e		e.Visits 2, 3, and 4 will be a telephone call only for subjects randomized to the control, untreated group. AE/SAE monitoring and concomitant medication review will be assessed during the call.	Added to clarify telephone call at Visits 2,3 and 4
Footnote j		in treated groups	Clarified subjects
Footnote k	~~To facilitate the additional BCVA measures this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF. The highest score will be used to define subject eligibility.~~		Moved text to end of footnote
		In Part II subjects, If …	Clarified subjects

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 8.0, 18 DEC 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		For all subjects who require triplicate BCVA testing, to facilitate the additional BCVA measures, this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF. At screening, The highest score will be used to define subject eligibility.	Text moved from beginning of footnote
Footnote m	~~Triplicate at every visit for Part II subjects only. Part I s~~	Subjects perform triplicate microperimetry only at baseline. Microperimetry should be conducted at unscheduled visits, if clinically feasible.	
Footnote q	~~Non-serious AEs will be collected from Visit 2 through Visit 10 (or ET Visit if applicable)~~	AEs/SAEs will be collected from the time the subject provides written informed consent/assent through Visit 10 (or ET Visit if applicable).	Added AEs to footnote and deleted collection of non-serious AEs
Footnote r		Corticosteroid review is applicable only for treated subjects.	Footnote was added to clarify subjects for review
Footnote t		, and only	Added to clarify procedure
Footnotes f-j	~~e., f., g., h., i., j., k., l., m., n., o., p., q., r., s.~~	f., g., h., i., j., k., l., m., n., o., p., q., r., s., t.	Updated footnote labels

SUMMARY OF CHANGES FOR CLINICAL STUDY PROTOCOL NSR-RPGR-01

AAV8-RPGR

A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

Indication:	X-Linked Retinitis Pigmentosa
Study Phase:	1/2/3
Sponsor:	NightstaRx Ltd 2nd Floor 10 Midford Place London W1T 5BJ, UK Telephone: +44 (0) 020 7062 2777
Summary of Changes:	Protocol Version 7.0 16 NOV 2018

CONFIDENTIALITY STATEMENT

SUMMARY OF CHANGES SPONSOR APPROVAL PAGE

Clinical Study Protocol Number:	NSR-RPGR-01
Protocol Title:	A Dose Escalation (Phase 1), and Dose Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)
Summary of Changes for Protocol:	Version 7.0 16 NOV 2018

Approved By:

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

[redacted] Date: 16-November-2018

[redacted], MD

Overview / Rationale: Changes to the Part II Dose-Expansion Phase of the Study

This protocol amendment changes the doses to be expanded in Part II from the "MTD and 3-dose levels lower than MTD" to dose 3 and dose 5. Rationale for this change was provided by the DMC at the October 2018 meeting. Although no DLTs have been identified through cohort 6 treated with the highest dose, and therefore a true MTD has not been identified in this dose-escalation, the committee recommended to expand dose 5 and dose 3, as the doses most likely to define safety and efficacy of AAV8-RPGR. In addition, regulatory agencies have recommended adding a third untreated arm to better establish efficacy and safety of the gene therapy. Thus the subjects will be randomized in a 1:1:1 allocation, with double masking to dose in the treated arms, and masked assessments of efficacy.

The study duration has been shortened to 12 months of follow-up for Part II dose-expansion because a long-term follow-up study will be initiated to continue follow-up of all AAV8-RPGR subjects from 12 months to out to 5 years. Phase 1 subjects will still be followed for 24 months, and then also invited to participate in the long-term study.

The endpoints and inclusion criteria are now separated by study Part to improve clarity.

An efficacy endpoint, microperimetry at 3 months, has been added to Part II based on early efficacy signals observed during dose-escalation. Related to this, an inclusion range for microperimetry has been added to Part II in order to assure the inclusion of subjects with modifiable disease and to avoid inclusion of those with microperimetry values subject to ceiling effects.

Additionally, to further our understanding of the effects of AAV8-RPGR, assessments of [redacted], and quality of life questionnaires have been added, the former two only in sites with the available technology. Some assessments (reading test, color vision, and full-field threshold sensitivity [FST]) have been removed from the study assessments due to the excessive burden of study visits on the subjects, and the limited value of these assessments in understanding both the disease and the effects of the drug.

To improve safety and to prevent potential inflammatory response to therapy, the steroid regimen has been modified. A standardized adult starting dose of 60 mg has been selected (rather than the previous 1mg/kg starting dose) and the taper has been lengthened to include coverage out to approximately 2 months. A 2-month visit has been added to assess the subjects at the end of the steroid therapy.

Information on the procedures related to unmasking has been added to comply with Good Clinical practices. Serious adverse event reporting has also been changed to immediately instead of within 7 days, per regional recommendations.

Page numbers on the following table of changes refer to the tracked changes version of the protocol amendment.

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Title Page	**A Dose Escalation, Phase ~~1/2~~ Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)**	A Dose Escalation (Phase 1), and Dose-Expansion (Phase 2/3) Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)	Added to title the dose-expansion and phase of the study has been specified for dose escalation to be Phase 1 and for dose expansion, to be Phase 2/3
Title Page, Pages 1, 5, 6, 22		STUDY PHASE 1/2/3	Added Phase 3 to Study Phase
Sponsor. Page 1	~~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	[redacted]	[redacted], MD	Personnel change
Contact Information. Pages 4-5	[redacted] ~~Rx Ltdr Wellcome Gibbs Building, Euston Road London NW1 2BE, UK +44 (0) 207 611 2034~~		The contact page within the protocol now includes only the pharmacovigilance contact and the responsible CRO. The complete study contact list will be included in the study manual. The safety email remains the same and is listed.
	[redacted] ~~NightstaRx Ltd Wellcome Gibbs Building, 215 Euston Road London NW1 2BE, UK +44 (0) 207 611 2193~~		
	[redacted] ~~, MD NightstaRx Ltd Wellcome Gibbs Building, 215 Euston Road London NW1 2BE, UK~~	[redacted] Email: [redacted]	Change in personnel and contact information
	Contract Research Organization [redacted] ~~UK~~	[redacted] UK	New address and phone

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	[redacted] ~~NightstaRx Ltd~~ ~~Wellcome Gibbs Building,~~ ~~215 Euston Road~~ ~~London NW1 2BE, UK~~ ~~781-457-+44 (0) 207 611 2271~~ ~~81 Hatrwell Avenue,~~ ~~Suite 100~~ ~~Lexington, MA 02421~~	[redacted]	The contact sheet within the protocol now includes only the pharmacovigilance contact and the responsible CRO. The complete study contact list will be included in the study manual. The safety email remains the same and is listed.
Synopsis Study Period (years) Page 5	~~36~~ months	Part I: 24 months Part II: 12 months	Duration of study corrected from estimate last patient, last visit to actual duration for each subject in Part I
Synopsis Endpoints Page 5	~~The primary safety endpoint is incidence of dose-limiting toxicities (DLTs) and treatment-emergent adverse events (TEAEs) over a 24-month period.~~	**Endpoints** **Part I** **Primary Endpoints:** The primary safety endpoints are the incidence of dose-limiting toxicities (DLTs), and treatment-emergent adverse events (TEAEs) over a 24-month period. Secondary and Exploratory Endpoints: • Change from baseline in microperimetry at 3, 6, 12, 18 and 24 months • Change from baseline in best-corrected visual acuity (BCVA) at 3, 6, 12, 18, and 24 months • Change from baseline in spectral domain optical coherence tomography (SD-OCT) at 3, 6,12, 18 and 24 months • Change from baseline in autofluorescence at 3, 6, 12, 18 and 24 months • Change from baseline in other anatomical and functional	Separated out Part I

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		outcomes at 3, 6, 12 months, 18 and 24 months	
Synopsis. Endpoints Pages 5-6		**Part II** **Primary Efficacy Endpoint:** The primary efficacy endpoint is improvement from Baseline in microperimetry at 3 months. **Safety Endpoint:** The safety endpoint is incidence of TEAEs over a 12-month period.	Separated out Part II
Synopsis. Endpoints Pages 5-6	• Change from baseline in microperimetry at ~~3,~~ 6, 9, 12~~ 18 and 24~~ months • Change from baseline in best-corrected visual acuity (BCVA) at 3, 6, 12~~ 18 and 24~~ months • Change from baseline in SD-OCT at 3, 6, 12 ~~18 and 24~~ months • Change from baseline in autofluorescence at 3, 6, 12,~~ 18 and 24~~ months	**Secondary Endpoints:** • Change from baseline in microperimetry at 1, 6, 9, and 12 months • Change from baseline in best-corrected visual acuity (BCVA) at 3, 6, and 12 months • Change from baseline in SD-OCT at 3, 6, and 12 months • Change from baseline in autofluorescence at 3, 6, and 12 months • Change from baseline in visual field assessed by Octopus 900 at 3, 6, and 12 months	Updated Part II endpoints
	• Change from baseline in other anatomical and functional outcomes at 3, 6, 12, ~~18 and 24~~ months	Exploratory Endpoints: • [redacted] • Change from baseline in adaptive-optics (AO)-OCT at 6 and 12 months • [redacted] • Change from baseline in other anatomical and functional outcomes at 3, 6, and 12 months	• Updated Part II endpoints
Synopsis. Study Design Pages 6-8	~~The study will be conducted in two parts: Part I is a dose escalation study, Part II is a Maximum Tolerated Dose (MTD) expansion study (as determined in Part I)~~	This is a Phase 1/2/3… Part I is a dose-selection study; Part II is a dose-expansion study, with 2 doses (2.5 × 10^11 gp [high dose], 5 × 10^10 gp [low dose]) selected from Part I based on a benefit/risk assessment, and a third untreated	Study design description updated to include Part I and Part II. Part II now defines doses as high and low doses and not as

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		group to allow for a controlled comparison of efficacy and safety.	MTD since the chosen high dose to be expanded has not been defined as MTD.
	The study will consist of visits over a 24-month evaluation period. At the Screening/Baseline Visit, each subject will be assessed for eligibility of both eyes. Only 1 eye will ~~receive treatment~~ (the "study eye"), and the ~~untreated~~ eye will be designated as the "fellow eye".	Part I consists of 12 visits over a 24-month evaluation period. Part II consists of 10 visits over a 12-month evaluation period. Only 1 eye will be randomized (the "study eye"), and the other eye will be designated as the "fellow eye". Selection of the "study eye" will be made on clinical grounds prior to randomization and will generally be the worse eye affected.	Differentiates the visit number and duration for Parts I and II
		Subjects in the untreated control group will receive study-visit telephone calls to monitor AEs/SAEs and review concomitant medications on Visit 2 (Day 0) and on Visits 3 and 4 (post-operative Days 1 and 7).	Information on conduct for untreated control subjects has been added regarding the surgery day and post-operative Days 1 and 7.
	To minimise inflammation resulting from surgery and/or vector/transgene, all subjects will be given a 21-day course of oral corticosteroid (e.g., prednisolone/prednisone) that will start 2 days before the planned date of surgery.	To minimise inflammation resulting from surgery and/or vector/transgene, in Part I, all subjects will be given a 21-day course of oral corticosteroid (e.g., prednisolone/prednisone) that will start 2 days before the planned date of surgery. In Part II, all subjects will be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), placed behind the globe via a deep sub-Tenon approach. All subjects will also be given a 9-week course of oral corticosteroid starting 3 days before surgery: 21 days at 60 mg, followed by 6 weeks of tapering doses. The dose regimen is adjusted for pediatric subjects treated in Part II (see Section 9.8).	To improve safety and to prevent potential inflammatory response to therapy, the steroid regimen has been modified. A standardized adult starting dose of 60 mg has been selected (rather than the previous 1mg/kg starting dose) and the taper has been lengthened to include coverage out to approximately 2 months. A 2-month visit has been added to assess the

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
			subjects at the end of the steroid therapy.
		At study completion, Part I subjects will be invited to participate in a separate long-term follow-up study that will collect efficacy and safety data up to 5 years from surgery.	Added the extension of follow-up for Part I subjects
	Part II: ~~MTD~~ Expansion ~~Study~~	Part II: Dose Expansion In Part II, 45 subjects will be randomized 1:1:1 to a high-dose (2.5 × 10^11 gp), a low-dose (5 × 10^10 gp), and a third untreated group to allow for a controlled comparison of efficacy and safety. Study data will be collected for both eyes of each subject. Since treatment requires an invasive surgical procedure under general anaesthesia, the sponsor, investigator and the subject will be unmasked to the study procedure (i.e., vitrectomy and sub-retinal injection), however within the treated groups, the sponsor, investigator and subject will be masked to the assigned dose. To further minimise potential bias of the treated and non-treated eye evaluations, all subjective ophthalmic assessments at the Screening/Baseline Visit (Visit 1) and from Month 1 (Visit 5) onwards will be conducted by a masked assessor.	Clarified randomization for Part II Dose Expansion as 3-armed study with untreated arm added, per general rationale above. Added language explaining the assessor masking.
	~~Once the MTD has been identified, up to 45 additional subjects will be randomized, in a 2:1 allocation ratio. Subjects will receive AAV8-RPGR either at the MTD (MTD cohort), or at a low dose (active control cohort), three dose levels below the MTD (e.g., low dose = 5 x 1010 gp if MTD = 5x1011 gp). Part II of the study will be randomized and double-masked to the assigned dose, and open-~~	Subjects, sponsor, investigators and clinical assessors will be masked to the assigned dose. All Part II subjects will be followed for 12 months with the pre-specified visit schedule. At study completion, Part II subjects will be invited to participate in a separate long-term follow-up study that will collect efficacy and safety data from Month 12 through 5 years from surgery.	Clarified randomization for Part II Dose Expansion as 3-armed study with untreated arm added, per general rationale above.

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~label to the treatment administration.~~		
Synopsis Inclusion Criteria Page 9	1. Subject ~~/ parent (if applicable)~~ is willing and able to give informed consent for participation in the study		Part I is only in adults so removed the language for minors
Synopsis Inclusion Criteria Page 9	2. Are male and able to comply and adequately perform all study assessments ~~Part I:≥18 years of age~~ ~~Part II: ≥10 years of age~~	Part I Are male, ≥18 years of age, and able to comply and adequately perform all study assessments.	Clarified age inclusion criterion for Part I; separated Part I and Part II inclusion criteria to add clarity for investigators;
Synopsis Inclusion Criteria Page 9	5. BCVA in both eyes that meets the following criteria, based on the cohort level: Cohort 4-6 ~~and study Part II~~: better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity)	5. BCVA in both eyes that meets the following criteria, based on the cohort level: Cohort 4-6: better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity)	Removed Part II BCVA inclusion criterion because inclusion criteria are now separated for Part II to improve clarity
Synopsis Inclusion Criteria Part II Pages 9-10		Part II Inclusion Criteria 1. Subject / parent (if applicable) is willing and able to provide informed consent for participation in the study 2. Are male, ≥10 years of age, and able to comply and adequately perform all study assessments 3. Have a genetically confirmed diagnosis of XLRP (with RPGR mutation) 4. Have active disease clinically visible within the macular region in the study eye and defined as follows: ➢ ellipsoid zone (EZ) on SD-OCT at screening must be measurable, and within the nasal and temporal border of any B-scan, and not be visible on the most inferior and superior B-scan	• Added section for Part II inclusion criteria to clarify the differences between Parts I and II; • EZ zone must be measurable on screening in the study eye • BCVA is same for all subjects in Part II (no cohort differences); specifies criterion is for study eye only • Added mean total retinal sensitivity range in the study

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		5.Have a BCVA in the study eye that meets the following criteria: •Better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity). 6. Mean total retinal sensitivity in the study eye as assessed by microperimetry ≥0.1 dB and ≤8 dB	eye to ensure that subjects have modifiable microperimetry not subject to ceiling effects
Synopsis, Exclusion Criteria Page 10		Exclusion Criteria: Parts I and II	Specifies that exclusion criteria are the same for both parts
Synopsis Test Product, Dosage, and Mode of Administration Page 10		Part I, Dose Escalation, Phase 1: All subjects will undergo vitrectomy and receive a single sub-retinal injection of AAV8-RPGR. Subjects will be assigned to 1 of the following AAV8-RPGR dose levels: 5 × 10^9 gp, 1 × 10^10 gp, 5 × 10^10 gp, 1 × 10^11 gp, 2.5 × 10^11 gp, or 5 × 10^11 gp. Part II, Dose Expansion, Phase 2/3: Subjects will be assigned to 1 of the following: high-dose (2.5 × 10^11 gp), low-dose (5 × 10^10 gp), or an untreated control arm.	Separated out Parts I and II for improved clarity
Synopsis. Reference Therapy Page 10	In Part I, no Reference therapies will be administered. In Part II, ~~a high dose and a low dose of AAV8-RPGR (active-control cohort) the concentration of which will be three dose levels from MTD.~~	In Part I, no Reference therapies will be administered. In Part II, 2 doses of AAV8-RPGR will be compared: a high-dose (2.5 × 10^11 gp) and a low-dose (5 × 10^10 gp). An untreated control arm will also be added.	Added comparator dosing and untreated arm to reference therapy
Synopsis. Criteria for Evaluation Page 10	**Efficacy:** The efficacy evaluation will be based on BCVA, SD-OCT, fundus autofluorescence, visual fields, microperimetry, contrast sensitivity, low luminance VA, ~~full field stimulus threshold test, colour vision and reading test.~~	**Efficacy:** The efficacy evaluation will be based on microperimetry (MAIA), BCVA, SD-OCT, [redacted], fundus autofluorescence, visual fields (Octopus 900 specified in Part II only), contrast sensitivity, LLVA, [redacted]	Efficacy evaluation updated to include MAIA microperimetry; for Part II only, [redacted] have been added; specifies Octopus 900 for visual field only in Part II

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Synopsis Statistical Methodology Page 11	The primary objective is to evaluate…	The primary objective of Part I is to evaluate…	Differentiates Part I objective from Part II objective
		The primary objective of Part II is to evaluate the efficacy and safety of AAV8-RPGR in an expanded population of subjects.	Differentiates Part I objective from Part II objective
	~~A sample size of 30 subjects at the MTD dose ensures that events with an incidence ≥10% will be identified with a 95% probability.~~	In Part II, subjects will be randomized 1:1:1 to the 2 doses of AAV8-RPGR: 2.5 × 10^11 gp and 5 × 10^10 gp or an untreated control group.	Randomization clarification for Part II, with addition of untreated control group
		Fisher's exact test will be utilized for the comparisons of binary endpoints, and T-test will be employed for the comparisons of continuous endpoints.	Statistical methods to include Fisher's exact test and T-tests
Section 6 Study Objectives and Endpoints Page 22	~~The primary safety endpoint is incidence of dose-limiting toxicities (DLTs) and treatment-emergent adverse events (TEAEs) over a 24-month period.~~	**Endpoints Part I Primary Endpoints:** The primary safety endpoints are the incidence of dose-limiting toxicities (DLTs), and treatment-emergent adverse events (TEAEs) over a 24-month period. Secondary and Exploratory Endpoints: • Change from baseline in microperimetry at 3, 6, 12, 18 and 24 months • Change from baseline in best-corrected visual acuity (BCVA) at 3, 6, 12, 18, and 24 months • Change from baseline in spectral domain optical coherence tomography (SD-OCT) at 3, 6,12, 18 and 24 months • Change from baseline in autofluorescence at 3, 6, 12, 18 and 24 months • Change from baseline in other anatomical and functional outcomes at 3, 6, 12 months, 18 and 24 months	Separated out Part I
Section 6.2.2 Study Objectives and Endpoints		**Part II Primary Efficacy Endpoint:**	Separated out Part II

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Page 22		The primary efficacy endpoint is improvement from Baseline in microperimetry at 3 months. **Safety Endpoint:** The safety endpoint is incidence of TEAEs over a 12-month period.	
Section 6.2.2.3 Study Objectives and Endpoints Page 19-20	• Change from baseline in microperimetry at ~~3,~~ 6, 9, 12~~ 18 and 24~~ months • Change from baseline in best-corrected visual acuity (BCVA) at 3, 6, 12~~ 18 and 24~~ months • Change from baseline in SD-OCT at 3, 6, 12 ~~18 and 24~~ months • Change from baseline in autofluorescence at 3, 6, 12,~~ 18 and 24~~ months	**Secondary Endpoints:** • Change from baseline in microperimetry at 1, 6, 9, and 12 months • Change from baseline in best-corrected visual acuity (BCVA) at 3, 6, and 12 months • Change from baseline in SD-OCT at 3, 6, and 12 months • Change from baseline in autofluorescence at 3, 6, and 12 months • Change from baseline in visual field assessed by Octopus 900 at 3, 6, and 12 months	Updated Part II endpoints
	• [redacted]	Exploratory Endpoints: [redacted]	Updated Part II endpoints
Section 7.1. Overall Design Pages 24-25	~~Part II is a Maximum Tolerated Dose (MTD) expansion study (as determined in Part I).~~	This is a Phase 1/2/3… The study will be conducted in two parts: Part I is a dose escalation study, Part II is a dose-expansion study, with 2 doses selected from Part I based on safety and efficacy, and a third untreated group to allow	Updated study design overview for Parts I and II to include expanded description of individual parts

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		for a controlled comparison of efficacy. Part I will identify the maximum tolerated dose (MTD) using a dose-escalation scheme. Part II will expand 2 doses, allowing for a broader assessment of the safety and efficacy of AAV8-RPGR with a larger sample size, including 45 subjects randomized 1:1:1 to the a high-dose, a low-dose, and an untreated arm. Part I primarily evaluates safety, defined by incidence of DLTs and TEAEs over a 24-month period. Phase II evaluates safety and efficacy, with inclusion of a primary efficacy endpoint, improvement from Baseline in microperimetry, evaluated at 3 months, and safety and secondary efficacy evaluated at 1-, 6-, 9- and 12 -months post-treatment. All Part II subjects treated under previous versions of the protocol will be followed for 12 months with the pre-specified visit schedule. At study completion, all subjects will be invited to participate in a separate long-term follow-up study that will collect efficacy and safety data up to 5 years from surgery.	
	~~The study will~~ consist of visits over a ~~24~~-month evaluation period.	Part I consists of 12 visits over a 24-month evaluation period. Part II consists of 10 visits over a 12-month period	Differentiated Part I and Part II visit number and duration
		To minimise inflammation resulting from surgery and/or vector/transgene, in Part I, all subjects will be given a 21-day course of oral corticosteroid. In Part II, all subjects will be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), placed behind the globe via a deep sub-Tenon approach. Subjects will also be given a 9-week course of oral corticosteroid starting 3 days before surgery: 21 days at 60 mg, followed by 6 weeks of tapering	• To improve safety and to prevent potential inflammatory response to therapy, the steroid regimen has been modified. A standardized adult starting dose of 60 mg has been selected

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		doses. The dose regimen is adjusted for pediatric subjects treated in Part II (see Section 9.8). Subjects in the untreated control group will receive study-visit telephone calls to monitor AEs/SAEs and review concomitant medications on Visit 2 (Day 0) and on Visit 3 and 4 (post-operative Days 1 and 7).	(rather than the previous 1mg/kg starting dose) and the taper has been lengthened to include coverage out to approximately 2 months. A 2-month visit has been added to assess the subjects at the end of the steroid therapy. • The treatment of untreated subjects at Visits 2, 3 and 4 has been added
	The efficacy evaluation will be based on BCVA, SD-OCT, fundus autofluorescence, ~~microperimetry,~~ visual fields, contrast sensitivity, low luminance visual acuity (LLVA), ~~full field stimulus threshold test (FST), colour vision, and reading test.~~ 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 surgery is performed, it should be carried out at least 4 weeks before Visit 1 (Year 1) ~~or Visit 11 (Year 2).~~ A subject is considered to have completed the study if he completes the ~~Year~~	The efficacy evaluation will be based on microperimetry, BCVA, SD-OCT, fundus autofluorescence, visual fields (Octopus 900 specified in Part II only), contrast sensitivity, low luminance visual acuity (LLVA), [redacted] and [redacted] Subjects who develop cataracts may undergo cataract surgery if deemed clinically necessary; if surgery is performed, it should be carried out at least 4 weeks before their final visit. A Part I subject is considered to have completed the study if he completes the Month-24 assessments. A Part II subject is considered to have completed the study if he completes the Month 12 assessments. The end of the trial is the date the last subject completes his final-visit 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.	• Clarified efficacy evaluations; added [redacted] at sites with available technology; added [redacted] • All above only in Part II • Specified Octopus 900 only in Part II for visual field assessment • Removed assessments that have caused unnecessary study burden in subjects and that have not provided valuable information in Part I • Differentiated duration in Part I

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~2~~ assessments. The end of the trial is the date the last subject completes his ~~Year 2~~ 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.		and Part II as well as definition of study completion
Section 7.1.3. Dose Expansion Page 27	~~Once the MTD has been identified, u~~Up to 45 additional subjects will be randomized, in a ~~2~~:1 allocation ratio^^ . ~~Subjects will receive AAV8-RPGR either at the MTD (MTD cohort), or at a low dose (active control cohort), three dose-levels below the MTD (e.g., low dose = 5 5 x x 1010 gp if MTD = 5x1011 gp).~~ ~~Part II of the study will be randomized and double-masked to the assigned dose, and open-label to the treatment administration.~~	Up to 45 additional subjects will be randomized in a 1:1:1 allocation ratio to a high-dose group (2.5 × 10^11 gp), a low-dose group (5 × 10^10 gp), and an untreated group. Study data will be collected for both eyes of each subject. Since treatment requires an invasive surgical procedure under general anaesthesia, the sponsor, investigator and the subject will be unmasked to the study procedure (i.e., vitrectomy and sub-retinal injection), however within the treated groups, the sponsor, investigator and subject will be masked to the assigned dose. To further minimise potential bias of the treated and non-treated eye evaluations, all subjective ophthalmic assessments at the Screening/Baseline Visit (Visit 1) and from Month 1 (Visit 5) onwards will be conducted by a masked assessor.	Part II description now identifies treatments as dose 5 and dose 3 and untreated arm added, per general rationale (see above). Description of assessor masking added
Section 7.3 Discussion of Study Design and Dose Selection Page 28	The planned sample size is consistent with a 3+3 escalation scheme. A prospective trial period of 24 months is considered to be a sufficient period of time to monitor for any AEs related to the vector and/or transgene/administration procedure.	The planned sample size in Part I is consistent with a 3+3 escalation scheme. A prospective trial period of 24 months is considered to be a sufficient period of time to monitor for any AEs related to the vector and/or transgene/administration procedure.	Specified Part I for dose escalation
Section 7.3 Discussion of Study	Further details are provided in the Investigator's Brochure.~~,~~	Further details are provided in the Investigator's Brochure.Given previous experience demonstrating	This information was removed as it

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Design and Dose Selection Pages 27-30	~~and a summary of the AAV8-RPGR doses in the toxicology species is presented in the table below. The safety and efficacy findings from other pre-clinical and clinical studies with AAV8 vector for subretinal delivery are also included for comparison.~~	the safety of higher subretinal doses of AAV8 vector (Vandenberghe 2011), and lacking safety signals at the lower range of doses, it should be possible to dose-escalate to the high end of the dose range of AAV8-RPGR	is redundant with the IB. The added language clarified the overall meaning.
	~~Table 1: Toxicology Safety Margin for Clinical Trials~~		Eliminated the table from the protocol as this is redundant with the IB.
	Once ~~the MTD has been identified~~ and the safety and tolerability of AAV8-RPGR is demonstrated in adults, subjects ≥10 year age will be enrolled in Part II of the study. The 10-years of age cut-off safeguards…	Once Part I dose-escalation has been completed, and the safety and tolerability of AAV8-RPGR is demonstrated in adults, subjects ≥10 years of age will be enrolled in Part II of the study. The 10-years of age cut-off safeguards…	Clarified that dose escalation completion is trigger to Part II
	In Part II, subjects will be randomized to '~~MTD cohort' or the 'active-control. The active-control cohort will be three dose-levels below the MTD. This assures a 1-1.5 log difference in dose between these two cohorts, and allows for identifying a dose response while mitigating the possibility of a subtherapeutic low dose.~~	In Part II, subjects will be randomized to a high-dose (2.5 × 10^11 gp), a low-dose (5 × 10^10 gp), and an untreated group. This allows for comparisons in a randomized and controlled fashion, as recommended by regulators for best practices in ophthalmic gene therapy studies (Human Gene Therapy for Retinal Disorders, Draft Guidance for Industry July 2018). The sponsor, investigator and the subject will be unmasked to the study procedure and treatment (i.e. vitrectomy and sub-retinal injection). However, within the treated groups, the sponsor, investigator and subject will be masked (i.e. double-masked) to the assigned dose. To further minimise potential bias of the treated and non-treated eye evaluations, all subjective ophthalmic assessments at the Screening/Baseline Visit (Visit 1) and from Month 1 (Visit 5)	Part II description now identifies treatments as dose 5 and dose 3 and untreated arm added, per general rationale (see above). Added description of masking procedures

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		onwards will be conducted by a masked assessor.	
Section 8.0 Selection and Withdrawal of Subjects Page 31		8.1 Part I	Differentiating Part I and Part II inclusion criteria to improve clarity
Section 8.1 Part I Section 8.1.1 Inclusion Criteria Page 31	Subject~~/ parent (if applicable)~~ is willing and able to give informed consent for participation in the study		• Part I is only in adults so removed the language for minors
Section 8.1 Part I Section 8.1.1 Inclusion Criteria Page 31	3. Are male and able to comply and adequately perform all study assessments ~~Part I:≥18 years of age~~ ~~Part II: ≥10 years of age~~	Part I Are male, ≥18 years of age, and able to comply and adequately perform all study assessments.	Clarified age inclusion for Part I; • separated Part I and Part II inclusion to add clarity for investigators;
Section 8.1 Part I Section 8.1.1 Inclusion Criteria Page 31	5. BCVA in both eyes that meets the following criteria, based on the cohort level: Cohort 4-6~~ and study Part II~~: better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity)	5. BCVA in both eyes that meets the following criteria, based on the cohort level: Cohort 4-6: better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity)	• Removed Part II because inclusion criterion is now separated for Part II to improve clarity
Section 8.2 Part II Section 8.2.1 Inclusion Criteria Page 31		Part II	• Differentiating Part I and Part II inclusion criteria to improve clarity

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 8.2 Part II Section 8.2.1 Inclusion Criteria Pages 31-32		Part II Inclusion Criteria 1. Subject / parent (if applicable) is willing and able to provide informed consent for participation in the study 2. Are male, ≥10 years of age, and able to comply and adequately perform all study assessments 3. Have a genetically confirmed diagnosis of XLRP (with RPGR mutation) 4. Have active disease clinically visible within the macular region in the study eye and defined as follows: ➢ ellipsoid zone (EZ) on SD-OCT at screening must be measurable, and within the nasal and temporal border of any B-scan, and not be visible on the most inferior and superior B-scan 5.Have a BCVA in the study eye that meets the following criteria: •Better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity). 6. Mean total retinal sensitivity in the study eye as assessed by microperimetry ≥0.1 dB and ≤8 dB	• Added section for Part II inclusion criteria to clarify the differences between Parts I and II; • EZ zone must be measurable on screening in the study eye • BCVA is same for all subjects in Part II (no cohort differences); specifies criterion is for study eye only • Added mean total retinal sensitivity range in the study eye to ensure that subjects have modifiable microperimetry not subject to ceiling effects
Section 8.3. Exclusion Criteria: Parts I and II Page 32		Parts I and II	To clarify that exclusion criteria are the same for Parts I and II
Section 9.1 Treatments Administered Page 34	~~At the Injection Day Visit (Visit 2, Day 0),~~ subjects will undergo vitrectomy and retinal detachment in their study eye and then receive a single, sub-retinal injection of AAV8-RPGR (See Section 9.4 for details).	Part I, Dose Escalation, Phase 1: all subjects will undergo vitrectomy and retinal detachment in their study eye and then receive a single, sub-retinal injection of AAV8-RPGR (See Section 9.4 for details). Part II, Dose Expansion, Phase 2/3: Subjects will be assigned to 1 of the following: high-dose (2.5 × 10^11 gp), low-dose (5 × 10^10 gp), or an untreated control arm.	Clarified the treatments in Part I and Part II
Section 9.3. Packaging, Labeling, Preparation and Storage Page 34	The Investigational Medicinal Product will be labelled in compliance with regulatory standards. ~~(on either the primary or secondary container) and~~	The Investigational Medicinal Product (IMP) will be labelled in compliance with regulatory standards.	Deleted label information.

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
	~~include the protocol study number, Sponsor's name, product name, titre, vial and lot number, expiration date, (IMP labellied for Europe only) storage conditions and caution statement.~~		
Section 9.4 Vitrectomy Procedure and Injection of AAV8-RPGR Pages 34-35		The subretinal injection technique to be used in this study is similar to that developed in the sponsor's choroideremia programme in Oxford… To date, over 150 subjects have been injected without complication by retinal surgeons using the technique described below. All subjects will be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), placed behind the globe via a deep sub-Tenon approach.	Updated vitrectomy procedure background and added description of additional local steroid administration at the time of surgery
Section 9.5 Randomisation Page 36	~~The~~ dose-escalation portion of this study is not randomized. In Part II, after the study eye is assigned, subjects will be randomised ~~a 2:1 ratioAAV8-RPGR MTD or a lower dose of AAV8-RPGR, three dose levels from MTD (e.g., low dose = 5 x 10^{10} gp if MTD = 5x10^{11} gp) for the active-control cohort.~~	Part I, the dose-escalation portion of this study, is not randomized. In Part II, after the study eye is assigned, subjects will be randomised to 1 of 3 groups with a 1:1:1 allocation ratio: 1) treatment with AAV8-RPGR at a high dose (2.5 × 10^11 gp); 2) treatment with AAV8-RPGR at a low dose (5 × 10^10 gp) or 3) no treatment.	Part II randomization procedure added; doses identified as dose 5 and dose 3 and untreated arm added, per introductory general rationale.
Section 9.6. Study Masking Page 36-37	Part II is ~~double~~ masked (subject, surgeon, investigator/site team, sponsor will be masked to the assigned dose, and open-label with respect to the treatment administration).	In Part II, all ophthalmic assessments that are conducted at the Screening/Baseline Visit will be conducted by appropriately qualified masked assessors. For the immediate post-operative visits, masking of the assessors will not be viable as clinical signs of surgery will be apparent (i.e. redness, swelling). Therefore, unmasked assessors will perform all ophthalmic assessments at Visit 3 (Day 1) and Visit 4 (Day 7). For Visit 5 (Month 1) onwards, masked assessors will be used, as any signs	Clarified masking procedure

		of surgery will have dissipated and it will not be possible clinically to differentiate between those subjects that have not undergone surgery, and those subjects that have undergone surgery and received active treatment. Subjects randomised to the untreated Control group will not be required to attend the site at Visit 2, 3 or 4. As the key purpose of Visit 2 is surgery, and Visit 3 and 4, post-operative safety, there is limited utility in Control subjects attending. Therefore, to limit the study burden for Control subjects thereby potentially reducing the risk of subject withdrawal at this stage and reducing the possibility of further unmasking due to direct contact and communication with fellow participants, Control subjects are not scheduled to attend the clinic for study visits at these times. In order to minimise bias further, masked assessors will not have access to the subject's medical records, source documentation or eCRF as data entries or notation (such as use of peri-operative corticosteroid) may be sources of unmasking. From Visit 5 (Month 1) onwards, the masked assessor will also read a pre-written statement to each subject, regardless of randomisation, reminding them of the masked nature of the study, and to avoid any reference to prior surgery/non-surgery, which eye may have received treatment or to allude to any information that may unmask the assessor as to which group the subject has been assigned to. Furthermore, it is anticipated that a subset of the subjects participating in the trial will be active on social media. Following appropriate approval by the Independent Ethics Committee (IEC)/Institutional Review Board (IRB), the patient information leaflet will request that subjects refrain from posting any details of study participation on social media, that may unmask the assessors to the group the subject has been assigned to. This request	

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		will be reiterated at subject visits by the investigator and within the pre-written statement. Subjects randomised to the AAV8-RPGR treatment groups, surgeons, the investigative team and the study sponsor will be masked to which dose of AAV8-RPGR the subject has been assigned to. Unmasked study site personnel will be assigned the responsibility of performing dilution, which will take place in a designated area remote from the investigative team to preserve masking of the treatment arm. Personnel delegated to perform the dilution will not be involved in any other aspect of the study (i.e., consent, safety/efficacy assessments, surgical procedure).	
Section 9.8 Concomitant Therapy Pages 37-39		To minimise inflammation, at the time of surgery, all subjects will be treated with a course of corticosteroid. In Part I, all subjects will also be prescribed a 21-day course of oral prednisone/prednisolone, following closely the 17-day protocol established in the Philadelphia AAV gene therapy clinical trial (Maguire et al., 2008), except allowing an extra 4 days for tapering the dose at the end of the course, i.e., 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. In Part II, at the time of surgery, all subjects (adult and pediatric) will be treated with up to 1 mL of triamcinolone, 40 mg/mL solution, which must be placed behind the globe via a deep sub-Tenon approach. In addition, all subjects	• To improve safety and to prevent potential inflammatory response to therapy, the steroid regimen has been modified. A standardized adult starting dose of 60 mg has been selected (rather than the previous 1mg/kg starting dose) and the taper has been lengthened to include coverage out to approximately 2 months. A 2-month visit has been added to assess the subjects at the end of the steroid therapy.

		will be prescribed a course of oral corticosteroids. For adults, 60 mg of oral prednisone / prednisolone will be prescribed for the initial 21 days (starting 3 days prior to surgery), followed by a weekly taper as follows for a total of 9 weeks of treatment: Day -3 through day 17 (21 days): 60 mg by mouth once daily Day 18 through day 24 (7 days): 50 mg by mouth once daily Day 25 through day 31 (7 days): 40 mg by mouth once daily Day 32 through day 38 (7 days): 40 mg by mouth once daily Day 39 through day 45 (7 days): 20 mg by mouth once daily Day 46 through day 52 (7 days): 10 mg by mouth once daily Day 53 through day 59 (7 days): 5 mg by mouth once daily. If at the Month-2 visit (Visit 5.9), inflammation is observed, corticosteroid therapy should be re-initiated, via oral and/or intraocular route, based on the clinical condition of the subject, and the judgement of the investigator. For pediatric subjects, oral prednisolone/prednisone will also be started 3 days prior to surgery. The starting dose will be based on kilogram weight of the subject, up to a maximum of 60 mg starting dose (rounded to the nearest 1 mg). Subsequent doses will have multipliers to provide the appropriate taper over an additional 6 weeks, for a total of 9 weeks of treatment. See tapering regimen for pediatric subjects below: Day -3 through day 17 (21 days): Starting Dose (SD) 1 mg/kg by mouth once daily (maximum dose of 60 mg/once daily) Day 18 through day 24 (7 days): SD X 0.83 mg by mouth once daily Day 25 through day 31 (7 days): SD X 0.67 mg by mouth once daily Day 32 through day 38 (7 days): SD X 0.5 mg by mouth once daily	• A pediatric regimen has also been added • General safety information regarding the use of steroids has been added

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		Day 39 through day 45 (7 days): SD X 0.33 mg by mouth once daily Day 46 through day 52 (7 days): SD X 0.17 mg by mouth once daily Day 53 through day 59 (7 days): SD X 0.08 mg by mouth once daily If at the Month-2 visit (Visit 5.9), inflammation is observed, corticosteroid therapy should be reinitiated, via oral and/or intraocular route, based on the clinical condition of the subject, and the judgement of the investigator. The local pediatric team should be involved with all children undergoing gene therapy surgery and should be available to give advice on the steroid doses used in each patient. Modification of the protocol-defined steroid treatment are allowed based on recommendations of the pediatric team with approval from the Sponsor. For all subjects, while taking oral steroids, special note should be made to follow for potential side effects (e.g., increased IOP, cataracts, hypertension, elevated blood sugar, infections, gastritis/peptic ulcer disease, edema, electrolyte imbalance, mood changes, insomnia), and appropriate prophylaxis and/or therapy should be instituted as needed (e.g., treatment with proton pump inhibitors and/or valcyclovir; restriction of nonsteroidal anti-inflammatory agents)	
Section 10.1 Visit 1 (Screening/Baseline Visit) Pages 38-39		The subject or parent will sign and date one copy of the consent form in the presence of the investigator or his/her designee; where applicable, an assent form will be completed by the subject.	• Moved for clarity

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 10.1 Visit 1 (Screening/Baseline Visit) Pages 40-41	• ~~RPGR mutation screen (only if not conducted previously)~~ •~~Colour vision test~~ •~~Speed reading test~~ •~~FST~~ Footnote: ~~It is recommended to measure BCVA and LLVA twice on the first day and once on the second day (prior to pupil dilation). All 3 BCVA and all 3 LLVA values must be recorded in the eCRF. The highest BCVA score will be used to define subject eligibility. LLVA should be conducted immediately after each BCVA assessment.~~	• For Part I, RPGR gene mutation screen (only if not conducted previously); for Part II, prior documentation of a non-synonymous mutation in the RPGR gene • ETDRS BCVA[1] • [redacted] • Fundus autofluorescence • MAIA Microperimetry[4] • Fundus photography • Octopus 900 visual fields[4,5] • Contrast sensitivity test • AE and SAE monitoring • [redacted] • [redacted] • Randomisation[3] **Footnotes:** 1. For Part I subjects, BCVA is performed in triplicate at baseline. For Part II subjects, if the BCVA value at Visit 1 (Screening/Baseline) is ≥ ± 10 letter gain or loss in the study eye compared to the previous XOLARIS study visit (if applicable), then BCVA must be repeated an additional 2 times, resulting in a total of 3 BCVA measures at Visit 1. To facilitate the additional BCVA measures this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF. The highest score will be used to define subject eligibility. If the BCVA value at Visit 1 (Screening/Baseline) is < ± 10 letter difference in the study eye compared to the previous XOLARIS study visit, then BCVA will be collected once and will not be repeated.	• Includes specifics on genetic diagnosis criterion • Assessments at Visit 1 now include [redacted] • Specifies Octopus 900 visual field • AE and SAE monitoring will occur from screening through end of study instead of SAE monitoring only • Removed reading test, colour vision and FST • Removed footnote information regarding BCVA and LLVA; added information in footnote related to Octopus 900 and new assessments • Added new information regarding baseline BCVA assessment requirements [redacted]

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		If subject was not previously in XOLARIS study, BCVA assessments at baseline must be triplicate. 2. Only at sites with available technology. To facilitate testing of [redacted] and [redacted], the visit can be conducted over 2 days. 3. [redacted] 4. Assessments collected in triplicate. To facilitate triplicate testing, the visit should be conducted over 2 days. Visual field and microperimetry outputs will be sent to a CRC for review. Data will be generated and collated within the CRC and exported to the Sponsor or designee for inclusion in the study database. 5. Octopus 900 perimetry is specified for visual field assessments only in Part II. [redacted]	[redacted] • Specified in footnote that Octopus 900 is only specified in Part II
Section 10.1 Visit 1 (Screening/Baseline Visit) Page 41	In Part II, subjects will be then randomised to one of the AAV8-RPGR treatment groups ~~(MTD cohort or active control cohort) and subjects receiving treatment will remain masked to the treatment dose.~~	In Part II, subjects will be informed of the randomisation outcome (i.e., AAV8-RPGR treatment or the Control group) and instructed to not reveal their treatment group assignment to the masked assessors during the study. Subjects randomised to the AAV8-RPGR treatment groups (along with the Investigators and sponsor) will remain masked to the assigned dose.	New randomization and treatments specified
Section 10.1 Visit 1 (Screening/Baseline Visit) Page 41	Subjects will be given a ~~21 day~~ course of oral prednisone/prednisolone	Subjects will be given a 21-day course of oral prednisone/prednisolone and instructed to start taking the drug 2 (Part I) or 3 (Part II) days before their next study visit (Visit 2).	Steroid course is no longer 21 days for all subjects
Section 10.2		At Visit 2, all subjects in the AAV8-RPGR groups will visit the surgical	• Ophthalmic exam has been added

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
(Day 0, Surgery/Injection Day Visit) Pages 41-42		site, and the following assessments will be performed prior to surgery: • Full ophthalmic examination, including indirect ophthalmoscopy, slit lamp examination with IOP assessment, anterior chamber and vitreous inflammation grading, and LOCS III cataract grading 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 analyses. All subjects will be treated at the time of surgery with up to 1 mL of triamcinolone (40 mg/mL), placed behind the globe via a deep sub-Tenon approach. Subjects in the Control group will receive a study visit Telephone Call at the agreed upon date and time. Sites will conduct the following assessments during the telephone call: • AE/SAE monitoring • Concomitant medication review.	• Language added because one arm is untreated in Part II • Added language specifying that other non-study procedures may be necessary but will not be collected • Added new local steroid therapy added at time of surgery • Added how the untreated arm will be assessed through a telephone call at that visit.
Section 10.3 Visit 3 (Day 1 Post-Operative Visit) Page 42-43		Subjects in the Control group will receive a study visit Telephone Call at the agreed upon date and time. Sites will conduct the following assessments during the telephone call: • AE/SAE monitoring • Concomitant medication review.	Added how the untreated arm will be assessed through a telephone call at that visit.
Section 10.4 Visit 4 (Day 7 Post-Operative Visit) Page 43		Subjects in the Control group will receive a study visit Telephone Call at the agreed upon date and time. Sites will conduct the following assessments during the telephone call: • AE/SAE monitoring	Added how the untreated arm will be assessed through a telephone call at that visit.

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		• Concomitant medication review.	
Section 10.5 Visit 5 (Month 1) Page 43		• MAIA Microperimetry	• Specified MAIA microperimetry
Section 10.6 Visit 5.9 (Month 2) Page 42		At Visit 5.9, the following assessments will be performed: • Collection of safety blood samples (haematology and clinical chemistry) • Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading • ETDRS BCVA • SD-OCT • Fundus autofluorescence • MAIA Microperimetry • Viral shedding • Immunogenicity sampling • AE/SAE monitoring • Concomitant medication review • Corticosteroid compliance review	New visit added to better monitor safety of subjects This visit is denoted as Visit 5.9 in order to maintain the visit numbering in the EDC
Section 10.7 Visit 6 (Month 3) Page 42-43	~~• Colour vision test~~ Footnote: ~~It is recommended to measure BCVA and LLVA twice on the first day and once on the second day (prior to pupil dilation). All 3 BCVA and all 3 LLVA values must be recorded in the eCRF. LLVA should be conducted immediately after each BCVA assessment.~~	• MAIA Microperimetry • Octopus 900 visual fields[1] [redacted] Footnotes: 1. Octopus 900 perimetry is specified for visual field assessments only in Part II. [redacted]	• Month 3 added new assessments; • removed color vision test • specified MAIA microperimetry • [redacted] • Specified in footnote that Octopus 900 is only specified in Part II • Removed footnote

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
			information in BCVA and LLVA
Section 10.8. Visit 7 (Month 6) Pages 45-46	• ~~Colour vision test~~ • ~~Speed reading test~~ • ~~FST~~	• [redacted] • MAIA Microperimetry • Octopus 900 visual fields[2] • [redacted] Footnotes: [redacted] 2. Octopus 900 perimetry is specified for visual field assessments only in Part II.	• Removed color vision, speed reading, and FST; • [redacted] • specifies MAIA microperimetry and Octopus 900 visual field
Section 10.9. Visit 8 (Month 9) Page 46	• ~~Fundus autofluorescence~~	• MAIA Microperimetry	• Removed autofluorescence; • MAIA microperimetry specified
Section 10.10. Visit 9 (Year 1) Pages 46-47	• ~~Colour vision test~~ • ~~Speed reading test~~ • ~~FST~~	• [redacted] • MAIA Microperimetry • Octopus 900 visual fields[2,3] • [redacted][1] • [redacted] Footnote: [redacted] 2. Octopus 900 perimetry is specified for visual field assessments only in Part II. [redacted] Subjects who develop cataracts may undergo cataract surgery if deemed clinically necessary; if surgery is performed, it should be carried out at least 4 weeks before Visit 9 (Year 1) or Visit 11 (Year 2) for Part II and Part I subjects, respectively.	• Removed color vision, reading and FST • [redacted], • Specified MAIA microperimetry and Octopus 900 visual field with associated footnotes • Differing times for Part I and Part II for window between potential cataract surgery and end of study visits • [redacted] • Specified in footnote that

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
			Octopus 900 is only specified in Part II
Section 10.11. Visit 10 (Month 18) Page 47		(Part I Subjects Only)	Specified that Visit 11, Month 18 is only for subjects of Part I
Section 10.12. Visit 11 (Year 2) Page 47-48	• ~~Colour vision test~~ • ~~Speed reading test~~ • ~~FST~~	(Part I Subjects Only)	• Specified that Visit 12, Month 24 is only for subjects of Part I • Removed assessments
Section 10.13 Early Termination (ET) Visit Page 48	• ~~Colour vision test~~ • ~~Speed reading test~~ • ~~FST~~	[redacted] • MAIA Microperimetry • Octopus 900 visual fields[2,3] • [redacted] Footnotes: [redacted] 2. Octopus 900 perimetry is specified for visual field assessments only in Part II. 3. Part II only.	• Added to ET visit, if possible, [redacted] MAIA Microperimetry, [redacted], and associated footnotes • Removed colour vision test, reading test, FST
Section 10.14 Unscheduled Visits Page 49		• Microperimetry, if clinically feasible	• Added microperimetry to unscheduled visit, if clinically feasible
Section 11.1 Best Corrected Visual Acuity Page 50	BCVA will be performed in triplicate over a 2-day period at Visits 1,9-and 11 (or ET Visit) for all subjects. It is recommended that BCVA will be conducted twice on the first day and once on the second day. All values will be entered in the eCRF.	For Part I subjects, BCVA will be performed in triplicate over a 2-day period at Visits 1, 9 and 11 (or ET Visit) for all subjects. It is recommended that BCVA will be conducted twice on the first day and once on the second day. All values will be entered in the eCRF. For Part II subjects, if the BCVA value at Visit 1 (Screening/Baseline) is ≥ ± 10 letter gain or loss in the study eye compared to the previous XOLARIS study visit (if applicable), then BCVA must be repeated an additional 2 times, resulting in a total of 3 BCVA measures at Visit 1. To facilitate the	New instructions on BCVA triplicate requirement

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		additional BCVA measures this visit should be conducted over 2 days, with BCVA measured twice on Day 1 and once on Day 2 (prior to pupil dilation). All 3 BCVA values must be recorded in the eCRF. The highest score will be used to determine subject eligibility. If the BCVA value at Visit 1 (Screening/Baseline) is < ± 10 letter difference in the study eye compared to the previous XOLARIS study visit, then BCVA will be collected once and will not be repeated. If subject was not previously in XOLARIS study, BCVA assessments at baseline must be performed in triplicate.	
[redacted]		[redacted]	[redacted]
Section 11.5 MAIA Microperimetry Page 51		MAIA Microperimetry will be conducted for both eyes at the times indicated in Table 3, Section 17.1. Microperimetry will be performed on both eyes in triplicate over a 2-day period at Visit 1 for all subjects. The final assessment should be used to determine subject eligibility. If at subsequent visits there are obvious technical challenges or the subject is not performing the assessment as expected from previous visits (e.g. distracted, large number of false positive responses, not maintaining fixation, etc), then the assessment may be repeated and	Specifies that MAIA microperimetry will be performed in triplicate at Visit 1 only or if there is an inconsistent reading at other visits

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		the second assessment should be used for that study visit.	
Section 11.6 Visual Fields Page 51		In Part II, visual fields should be assessed using the Octopus 900 perimeter.	Specifies that Octopus 900 visual field will be conducted during Part II.
Section 11.7. Contrast Sensitivity Page 52	~~Contrast sensitivity will be measured prior to pupil dilation using a Pelli Robson chart.~~		No longer specifies using the Peli-Robson chart.
Section 11.8 Low Luminance Visual Acuity Page 52	The test should be performed ~~after~~ before BCVA testing and ~~prior to~~ pupil dilation. ~~LLVA should be performed in triplicate over a 2-day period at Visit 1 and , Visit 9 8 (Month 3) and Visit 11 (1 Year) (or the ET Visit) for all subjects, if applicable. It is recommended that LLVA will be conducted twice on the first day and once on the second day. All values will be entered into the eCRF.~~	The test should be performed ~~-~~before BCVA testing and pupil dilation.	LLVA is no longer conducted in triplicate; it is now conducted prior to BCVA testing instead of after
Full Field Stimulus Threshold Test Page 52	~~FST will be measured for both eyes after a period of dark adaptation and at the times indicated in Table 3, Section 17.1 (Visit 1, 7, 9, 11, ET) only at sites where the required FST equipment is available, as specified in the Study Operations Manual. FST measurements will be taken by appropriately qualified technicians. For complete technical specifications, refer to the Study Operations Manual.~~		Removed assessment

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Colour Vision Page 52	~~Colour vision will be tested for both eyes prior to pupil dilation, at the times indicated in Table 3, Section 17.1. Eyes will be tested separately and in the same order at each assessment. For colour vision testing, assessors will be appropriately qualified for conducting the assessment.~~		Removed assessment
Reading Test Page 52	~~Reading performance will be evaluated prior to pupil dilation for both eyes at the times indicated in Table 3, Section 17.1. The reading test will be provided to each site by the Sponsor; for complete user instructions, refer to the Study Operations Manual. For the reading test, assessors will be appropriately qualified for conducting the assessment.~~		Removed assessment
[redacted]		[redacted]	[redacted]
[redacted]		[redacted]	[redacted]

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		[redacted]	
Section 12.2.2 Recording of Adverse Events Page 57		AEs/SAEs… AEs/SAEs that the investigator becomes aware of, and which are deemed to have a relationship to the study drug should continue to be reported to NightstaRx following the completion of the study for a period of up to 5 years following surgery, until/unless the subject is enrolled in another NightstaRx study.	AEs and SAEs will be collected from the time of informed consent to the end of study. New sentence added to mandate collection of AE information from subjects for up to 5 years from the end of study
Section 12.2.4 Reporting of Serious Adverse Events and DLTs Page 57	All cases that are fatal or life-threatening will be reported ~~no later than 7 days~~ after the sponsor received the initial report from the Investigator.	The sponsor may unmask any SAE reports that are serious, unexpected, and related to the study drug, as required, in accordance with safety reporting guidance and regulations. All cases that are fatal or life-threatening will be reported immediately after the sponsor receives the initial report from the Investigator.	Added language regarding unmasking per GCPs Shortened window for reporting SAEs per regional regulators
Section 12.2.5 Procedures for Unmasking Page 57		Procedures for Unmasking The Investigator has the ability to unmask an individual subject's treatment assignment, but this capability is restricted to medical emergencies in which knowledge of the subject's treatment is critical to the Investigator's ability to treat the subject. In such a case, the Investigator must first attempt to contact the Medical Monitor or backup to discuss the rationale for unmasking. If the Investigator determines that the conditions above are met, then s/he unmasks the subject through the	New section added per GCPs regarding unmasking procedures

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		Electronic Data Capture (EDC) system, files the resulting confirmation in a restricted file until the end of the study, and notifies the Medical Monitor within 24 hours that the subject's treatment assignment has been unmasked. The Investigator will not communicate the treatment assignment to the Medical Monitor or to any sponsor or study team staff; nor should the treatment assignment be communicated to site staff other than those personnel who require that knowledge in order to treat the subject. The Investigator will ensure that the rationale, date, and time of unmasking is noted in the subject's source documentation, but not the treatment assignment. The medical emergency that necessitated unmasking must also be recorded in the source documentation and the case report form per standard study procedures.	
Section 13.1 Sample Size Page 60	A sample size of ~~30~~ subjects ~~at the MTD dose~~ ensures ~~that events with an incidence ≥10% will be identified with a 95% probability.~~	Due to the nature of the study design of Part I, no formal sample size computation was performed. In Part II, A a sample size of 45 subjects, 15 in each of 3 groups (high-dose, low-dose and untreated), ensures 80% power at 0.05 significance level assuming that the treated arm has 50% probability of achieving ≥7 dB improvement at ≥5 loci at Month 3 vs. 5% in the untreated control arm.	Specified Part I and Part II sample size rationale
Section 13.3.1 Safety Analysis Set Page 60		The Safety Analysis Set will consist of all subjects who receive study treatment (vitrectomy/AAV8-RPGR) in Part I, and all subjects that are randomized in Part II.	
Section 13.3.2 Full Analysis Set Page 60	The Full Analysis Set will include all subjects for whom data of at least 1 post baseline efficacy assessment is available in ~~at least one~~ eye.	The Full Analysis Set will include all subjects for whom data of at least 1 post baseline efficacy assessment is available in the study eye.	

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 13.4 Descriptive Statistics Page 60		No formal statistical comparison will be performed in Part I, and statistical tests will be conducted for the efficacy endpoints in Part II.	
	Summaries will be generated by dose and overall, in Part I and, by group (~~MTD~~ dose, ~~and~~ low-dose) in Part II.	Summaries will be generated by dose and overall, in Part I and, by group (high-dose, low-dose, untreated) in Part II.	Included untreated arm
Section 13.5 Demographics and Baseline Characteristics Page 61		Demographics and baseline ocular characteristics will be summarised for the safety analysis set and the full analysis set for Part I and Part II separately.	
Section 13.6 Efficacy Analyses Page 61	Efficacy assessments are ocular in nature and therefore will be tabulated by eye (Study Eye and Fellow Eye). Efficacy data will be summarised using descriptive statistics (described in Section 13.4). ~~Change~~ from baseline in ~~BCVA~~ will be tabulated by visit and by eye~~.~~	Efficacy assessments are ocular in nature and therefore will be tabulated by eye (Study Eye and Fellow Eye). Efficacy data will be summarised using descriptive statistics (described in Section 13.4). This will be done for each cohort in Part I, and each arm in Part II. Improvement in retinal sensitivity and change from baseline in retinal sensitivity will be tabulated by visit and by eye in each Part, and be compared between the randomized groups in Part II by visit for the study eye.	
Section 13.6.1 Multiplicity Adjustment Page 61	~~Alpha adjustment is not applicable in this exploratory Phase 1/2 study~~ study.	No multiplicity adjustment is planned in the study. Part I is the dose-escalation to identify the MTD based on the evaluation of benefit and risk without any formal statistical comparisons, and therefore no multiplicity adjustment is needed. In Part II, the analysis at 3 months for the primary efficacy endpoint will determine whether the study is positive or not. Each dose will be compared with the untreated control separately by Fisher's Exact Test at 0.05 level (two-sided).	Specifies Part I and Part II multiplicity adjustment requirements
Section 13.8 Interim Analyses Page 62	~~In Part II, secondary endpoints will be analysed at 3, 6, 12, 18 and 24 months with masking to treatment dose maintained.~~	In Part II, the analysis after all subjects complete 3-month visit will evaluate the primary efficacy endpoint, and hence is not an interim analysis. The personnel who are involved in daily operation will	

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
		continued to be masked of the treatment information until study completion to minimize any operational bias. Additional analysis and a final analysis will be conducted for regulatory interactions.	
Section 14.1 Informed Consent Page 63		This is an assessor-masked study. All subjects will be informed at the time of randomisation whether they have been randomised to the AAV8-RPGR or Control group. However, subjects in the AAV8-RPGR treatment groups will be masked to the assigned dose.	Masking and randomization information have been added
Section 15.2 Data Handling and Records Management Page 65	~~(by KCT Data).~~ ~~(by KCT Data).~~		Removes specification for KCT Data
Section 15.4 Time and Schedule of the Study Page 66	~~The expected overall study duration is 36 months.~~	Part I includes 24 months of follow-up for each subject enrolled and treated. Part II includes 12 months of follow-up for each subject enrolled and treated.	Duration changed to 24 months for overall study duration of Part 1. This has been corrected to define the actual duration per subject and not the estimated duration to last patient last visit. Part II is defined similarly, i.e., with 12 months of follow-up for each subject.
Section 17.1 Schedule of Study Procedures Page 69-71			• Changes reflect changes in assessments detailed above under individual visits • Abbreviations updated • Footnotes updated

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 7.0, 16 NOV 2018 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Section 17.1 Schedule of Study Procedures Page 72-75	a. ~~Subjects will take 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).~~	Footnotes	Removed details on steroid regimen. This information can be found in body of protocol.

SUMMARY OF CHANGES FOR CLINICAL STUDY PROTOCOL NSR-RPGR-01

AAV8-RPGR

A Dose Escalation, Phase 1/2 Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

Indication:	X-Linked Retinitis Pigmentosa
Study Phase:	1/2
Sponsor:	NightstaRx Ltd Wellcome Gibbs Building, 215 Euston Road London NW1 2BE, UK Telephone: +44 (0) 207 611 2077
Summary of Changes:	Protocol Version 6.0 18 MAY 2018

CONFIDENTIALITY STATEMENT

SUMMARY OF CHANGES SPONSOR APPROVAL PAGE

Clinical Study Protocol Number:	NSR-RPGR-01
Protocol Title:	A Dose Escalation, Phase 1/2 Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)
Summary of Changes for Protocol:	Version 6.0 18 MAY 2018

Approved By:

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

[redacted]_____Date: 18/5/18

[redacted] MD

[redacted]

Overview / Rationale

This protocol amendment allowed for an expansion of an active-control cohort of subjects to occur concurrently with the expansion of the MTD cohort in a randomized, double-masked fashion. This change provides greater efficacy information through a comparison of low- and high-dose outcomes. The amendment also clarifies the consent procedures for subjects less under 10 years of age, and removes assessments of low luminance best-corrected visual acuity, blood pressure, pulse, and safety blood sampling at various visits. The primary endpoint has been clarified regarding the incidence of dose-limiting toxicities (DLTs) and adverse events, and the secondary endpoints have been specified temporally. Lastly, cohorts 5 and 6, which originally were optional, are now protocol-defined due to a lack of DLTs observed in the ongoing study in cohorts 1-4. Per the protocol, if DLTs are observed at cohort 5, no escalation to cohort 6 will occur.

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
Sponsor Approval Page 2	[redacted] ~~MD~~	[redacted], MD	Designated Sponsor approval has changed
Contact Information Page 4	[redacted]	[redacted]	Personnel change
	----	[redacted] PhD	New personnel added
Synopsis Primary Endpoint Page 5	The primary endpoint~~ is the assessment of safety and tolerability of a single sub-retinal injection of AAV8 RPGR in subjects with a genetically confirmed XLRP, due to retinitis pigmentosa GTPase regulator (RPGR) mutation,~~ over a 24-month period.	The primary safety endpoint is incidence of dose-limiting toxicities (DLTs) and treatment-emergent adverse events (TEAEs) over a 24-month period.	Clarified endpoint of analysis
Synopsis Secondary Endpoints Page 5	• Changes from baseline in spectral domain optical coherence tomography (SD-OCT) • Changes from baseline in best corrected visual acuity (BCVA) • Changes from baseline in autofluorescence • Changes from baseline in microperimetry	• Changes from baseline in microperimetry at 3, 6, 12, 18 and 24 months • Changes from baseline in best-corrected visual acuity (BCVA) at 3, 6, 12, 18, and 24 months • Changes from baseline in spectral domain optical coherence tomography (SD-OCT) at 3, 6, 12, 18 and 24 months	Added time points for precision of analyses; changed order

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
		• Changes from baseline in autofluorescence at 3, 6, 12, 18 and 24 months	
Synopsis Exploratory Endpoint Page 5	[redacted]	[redacted]	[redacted]
Synopsis Study Design Page 5-6	This is a ~~multi-centre, open-label, single ascending dose~~ interventional study of AAV8-RPGR in ~~adult~~ male subjects with genetically confirmed XLRP.	This is a Phase 1/2, first-in-human, multi-centre, dose-escalation interventional study of AAV8-RPGR in male subjects with genetically confirmed XLRP. The study will be conducted in two parts: Part I is a dose escalation study, Part II is a Maximum Tolerated Dose (MTD) expansion study (as determined in Part I).	The study design description now includes Part I and Part II
	Only 1 eye will receive treatment (the "study eye"), with the ~~contra-lateral~~ eye ~~serving as the control~~ (the "~~control~~ eye")	Only 1 eye will receive treatment (the "study eye"), and the untreated eye will be designated as the "fellow eye".	More clarity around the definition of the fellow eye
	~~The study will use a~~ 3+3 escalation scheme (Storer, 1989) for administration of AAV8-RPGR. Initially, the study will involve up to 4 dose cohorts, with AAV8-RPGR doses of 5×10^9 genome particles (gp) (Cohort 1), 1×10^{10} gp (Cohort 2), 5×10^{10} gp (Cohort 3) and 1×10^{11} gp (Cohort 4).	Part I: Dose-Escalation Study Dose escalation part of the study will use a 3+3 escalation scheme (Storer, 1989) and will involve up to 6 AAV8-RPGR dose cohorts: 5×10^9 genome particles (gp) (Cohort 1), 1×10^{10} gp (Cohort 2), 5×10^{10} gp (Cohort 3), 1×10^{11} gp (Cohort 4), 2.5×10^{11} gp (Cohort 5), and 5×10^{11} gp (Cohort 6).	Cohorts 5 and 6 have been added to the dose-escalation scheme;
	Dose limiting toxicities (DLTs) are defined as any of the following events considered to be related to AAV8-RPGR: • Sustained decrease in BCVA of ≥30 letters on the Early Treatment of Diabetic Retinopathy Study	Same text moved to the sixth paragraph of Study Design.	To clarify the two parts of the study, DMC and DLT definitions have been moved to the end of the Study Design description

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
	(ETDRS) chart compared to baseline. Sustained is defined as lasting 48 hours or more until recovery, with recovery defined as visual acuity (VA) returning to within 10 letters of baseline VA. An exception is made for surgery-related events occurring in close temporal association (within <24 hours) of the surgery. • Vitreous inflammation, vitritis (>Grade 3 using standardised Nussenblatt vitreous inflammation scale grading) (Nussenblatt et al., 1985). • Any clinically significant retinal damage observed (e.g., retinal atrophy) that is not directly attributed to complications of surgery. Any clinically relevant suspected unexpected serious adverse reaction (as defined in the Investigator's Brochure), with the exception of vision loss or vision-threatening events (as defined in Section 12.2.1.2).		
Synopsis Study Design **Page 6**	An independent Data Monitoring Committee (DMC) will review safety data before confirming whether escalation to a higher dose level can occur. There is a potential for surgical complications resulting in safety events that meet the criteria for a DLT. In such cases, the DMC will make the final adjudication as to whether the event is a DLT. The DMC will review safety data for each cohort when at	Same text moved to the fifth paragraph of Study Design	To clarify the two parts of the study, DMC and DLT definitions have been moved to the end of the Study Design description.

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
	least 3 subjects have been dosed at a particular level. However, if 2 subjects within a cohort have a DLT(s), dosing will not proceed to subsequent subjects until safety data are reviewed by the DMC. For the purpose of making decisions regarding dose escalation, the DMC will review safety data collected for at least 4 weeks from each subject in the last dosed cohort. In addition, the DMC will review cumulative safety data collected from all previously-dosed cohorts and take these findings into consideration when making decisions on dose escalation.		
Synopsis Study Design Page 6	If ≥2 subjects within a cohort (3 or 6 subjects) have a DLT(s), then the maximum tolerated dose (MTD) will be identified as the previous (lower) dose, ~~and 15 to 30 additional subjects will be treated at the MTD (MTD cohort)~~	If ≥2 subjects within a cohort (3 or 6 subjects) have a DLT(s), then the maximum tolerated dose (MTD) will be identified as the previous (lower) dose.	Addition of Part II description provides this information in more detail with active-control cohort.
	~~If subjects in Cohorts 1-4 tolerate treatment, the sponsor and DMC will review all cumulative safety data and determine if further dose escalation (2.5×10^{11} gp [Cohort 5], and subsequently 5×10^{11} gp [Cohort 6]) is acceptable. If dose escalation is considered to be feasible the same procedure described above for Cohorts 1-4 will be followed. If dose escalation is not deemed feasible, then 15 to 30 additional subjects will be treated at the defined MTD.~~		Text removed. Cohorts 5 and 6 are now protocol-specified. Expansion is now described in Part II.
		Part II: MTD Expansion Study	New text describes Part II.

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
		Once the MTD has been identified, up to 45 additional subjects will be randomized, in a 2:1 allocation ratio. Subjects will receive AAV8-RPGR either at the MTD (MTD cohort), or at a low dose (active-control cohort), three dose-levels below the MTD (e.g., low dose = 5 x 1010 gp if MTD = 5x1011 gp). Part II of the study will be randomized and double-masked to the assigned dose, and open-label to the treatment administration.	
Synopsis, Number of Subjects Page 6	~~In a 4 cohort trial, it is anticipated that up to 42 subjects will be enrolled, dependent on toxicity observed.~~	Overall, the study is expected to enroll approximately 63 subjects: 18 in Part I and 45 in Part II.	Numbers are increased for Cohorts 5 and 6 in Part I and for expansion with active-control cohort in Part II.
Synopsis, Diagnosis and Inclusion Criteria Page 7	Are willing and able to give informed consent for participation in the study	Subject / parent is ~~Are~~ willing and able to give informed consent for participation in the study	Parent is included in all informed consent language for inclusion of pediatric subjects.
	Are male and able to comply and adequately perform all study assessments • Cohorts 1-4 (5 and 6, if applicable): ≥18 years of age • MTD cohort: ≥10 years of age	Are male and able to comply and adequately perform all study assessments • Study Part I: ≥18 years of age • Study Part II: ≥10 years of age	States more clearly the two parts of the study with age cut-offs
	Have active disease clinically visible within the macular region.	Have active disease clinically visible within the macular region in both eyes and defined as follows: ➢ ellipsoid zone (EZ) on SD-OCT measured at screening, must be within the nasal and temporal border of any B-scan, and not be visible on the most inferior and superior B-scan	Allows for a more specific definition of the disease in at least one eye, with anatomical characteristics that might demonstrate improvement with interventions

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
	BCVA in both eyes that meets the following criteria, based on the cohort level: ○ Cohort 4 ~~(5 and 6, if applicable) and MTD cohort~~: better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity)	BCVA in both eyes that meets the following criteria, based on the cohort level: ○ Cohort 4-6 and study part II: better than or equal to BCVA of 34 ETDRS letters (equivalent to better than or equal to 6/60 or 20/200 Snellen acuity)	
Synopsis, Exclusion Criteria **Page 8**	Are unwilling to use barrier contraception methods for a period of 3 months following treatment with AAV8-RPGR	Are unwilling to use barrier contraception methods (if applicable), for a period of 3 months following treatment with AAV8-RPGR	Inclusion of pediatric subjects
Synopsis, Test Product, Dosage, and Mode of Administration **Page 8**	Subjects will be assigned to 1 of the following ~~potential~~ cohorts: • ~~Cohort 1:~~ 5×10^9 gp/~~0.1~~ mL • ~~Cohort 2:~~ 1×10^{10} gp/~~0.1~~ mL • ~~Cohort 3:~~ 5×10^{10} gp/~~0.1~~ mL • ~~Cohort 4:~~ 1×10^{11} gp/~~0.1~~ mL ~~MTD: As identified from Cohorts 1-4.~~	Subjects will be assigned to 1 of the following AAV8-RPGR dose cohorts: 5×10^9 gp/mL, 1×10^{10} gp/mL, 5×10^{10} gp/mL, 1×10^{11} gp/ mL, 2.5×10^{11} gp/mL, or 5×10^{11} gp/mL.	Addition of cohorts 5 and 6; Addition of cohorts 5 and 6 to protocol specified instead of optional if no DLTs are observed; error corrected in concentration / mL
Synopsis, Reference Therapy	~~The contralateral eye will serve as a comparator for efficacy in this study. The contralateral eye will not undergo surgery or receive any study treatment.~~	In Part I, no Reference therapies will be administered. In Part II, a low dose of AAV8-RPGR (active-control cohort) will be the Reference therapy, the concentration of which will be three dose levels from MTD.	Separation of Part I and II as there is an active-control cohort in Part II
Synopsis, Statistical Methodology	~~Summaries will be generated by dose and overall.~~ AEs will be summarised by system organ class and preferred term. Both the number of eyes/subjects experiencing an AE and the number of events will be summarised. Similar	The primary objective of the study is to evaluate the safety profile of AAV8-RPGR. The primary safety endpoint is incidence of DLTs and TEAEs over a 24-month period. The analysis population for the safety analysis will include all	Statistical methodology is specified.

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
	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. ~~Separate summaries will be generated for ocular and systemic AEs.~~ Summary statistics will be presented for both eyes (Study Eyes ~~versus Control Eyes).~~	patients enrolled in the study who received AAV8-RPGR treatment. A sample size of 30 subjects at the MTD dose ensures that events with an incidence ≥10% will be identified with a 95% probability. [redacted] Summary statistics will be presented for both eyes (Study Eye and Fellow Eye). (text continues unchanged) A by-subject listing of SAEs and DLTs will be provided.	
Section 6 **6.2.1 Primary Endpoint**	The primary endpoint is ~~the assessment of safety and tolerability of a single sub-retinal injection of AAV8-RPGR in subjects with genetically confirmed XLRP, due to an RPGR mutation,~~ over a 24-month period.	The primary safety endpoint is incidence of dose-limiting toxicities (DLTs) and treatment-emergent adverse events (TEAEs) over a 24-month period.	Clarified the endpoint
6.2.2 Secondary Endpoints	• Changes from baseline in spectral domain optical coherence tomography (SD-OCT) • Changes from baseline in best-corrected visual acuity (BCVA) • Changes from baseline in autofluorescence • Changes from baseline in microperimetry. • Changes from baseline in other anatomic and functional outcomes.	• Changes from baseline in microperimetry at 3, 6, 12, 18, and 24 months • Changes from baseline in best-corrected visual acuity (BCVA) at 3, 6, 12, 18, and 24 months • Changes from baseline in spectral domain optical coherence tomography (SD-OCT) at 3, 6, 12, 18, and 24 months • Changes from baseline in autofluorescence at 3, 6, 12, 18, and 24 months • Changes from baseline in other anatomic and	Added specific time points for analyses; changed order

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
		functional outcomes at 3, 6, 12, 18 and 24 months	
7.1 Overall Study Design	This is a multi-centre, ~~open-label, single ascending dose~~ interventional study of AAV8-RPGR in adult male subjects with genetically confirmed XLRP.	This is a Phase 1/2, first-in-human, multi-centre, dose-escalation interventional study of AAV8-RPGR in male subjects with genetically confirmed XLRP. The study will be conducted in two parts: Part I is a dose escalation study, Part II is a Maximum Tolerated Dose (MTD) expansion study (as determined in Part I).	
7.1 Overall Study Design	Only 1 eye will receive treatment (the "study eye"), ~~with the contra-lateral eye serving as the control~~ (the "~~control~~ eye").	Only one eye will receive treatment (the "study eye"), and the untreated eye will be designated as the "fellow eye."	Clearer definition of untreated eye
7.2 Dose-Limiting Toxicities	…. In the event that a DLT occurs at any time during the study, the site is to report the event to the sponsor within 24 hours of occurrence.	When triplicate BCVA assessments are performed at screening, the median BCVA result will be used for change-from-baseline BCVA computation. In the event that a DLT occurs at any time during the study,…	Clarified change from baseline computation for DLTs when triplicate assessments are performed
7.3 Dose Escalation	7.3 Dose Escalation Scheme	7.3 Part I: Dose-Escalation Study	Changed Header to reflect Part I of study
	~~Initially,~~ the study will involve up to 4 dose cohorts, with AAV8-RPGR doses of 5×10^9 gp (Cohort 1), 1×10^{10} gp (Cohort 2), 5×10^{10} gp (Cohort 3), and 1×10^{11} gp (Cohort 4).	The study will involve up to 6 dose cohorts, with AAV8-RPGR doses of 5×10^9 gp (Cohort 1), 1×10^{10} gp (Cohort 2), 5×10^{10} gp (Cohort 3), and 1×10^{11} gp (Cohort 4), 2.5×10^{11} gp (Cohort 5), and 5×10^{11} gp (Cohort 6).	Addition of cohorts 5 and 6 to protocol specified instead of optional if no DLTs are observed
	If ≥2 subjects within a cohort (3 or 6 subjects) have a DLT(s), then the maximum tolerated dose (MTD) will be identified as the previous (lower) dose~~, and an additional 15 to 30 subjects~~	If ≥2 subjects within a cohort (3 or 6 subjects) have a DLT(s), then the maximum tolerated dose (MTD) will be identified as the previous (lower) dose.	

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
	~~will be treated at the MTD (MTD cohort)~~.		
7.4 Part II: MTD Expansion Study		PART II: MTD EXPANSION STUDY Once the MTD has been identified, up to 45 additional subjects will be randomized, in a 2:1 allocation ratio. Subjects will receive AAV8-RPGR either at the MTD (MTD cohort), or at a low dose (active-control cohort), three dose-levels below the MTD (e.g., low dose = 5 x 1010 gp if MTD = 5x1011 gp). Part II of the study will be randomized and double-masked to the assigned dose, and open-label to the treatment administration.	
7.5 Number of Subjects	~~In a 4-cohort trial, it is anticipated that up to 42 subjects will be enrolled, dependent on toxicity observed.~~	Overall, the study is expected to enroll approximately 63 subjects: 18 in Part I and 45 in Part II. See Section 13.1 for rationale.	
7.6 Discussion of Study Design and Dose Selection	Once the safety and tolerability of AAV8-RPGR is demonstrated in adults, subjects ≥10 years of age will be enrolled in the MTD cohort.	Once the MTD has been identified and the safety and tolerability of AAV8-RPGR is demonstrated in adults, subjects ≥10 years of age will be enrolled in Part II of the study.	
7.6 Discussion of Study Design and Dose Selection		In Part II, subjects will be randomized to the 'MTD cohort' or the 'low-dose active-control cohort'. This allows for a parallel, active-control group and masking of the treatment dose, which will enhance the robustness of the efficacy and safety outcomes. The active-control cohort will be three dose-levels below the MTD. This assures a 1-1.5-log difference in dose between these two cohorts, and allows for identifying a dose	

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
		response while mitigating the possibility of a subtherapeutic low dose.	
8.1 Inclusion Criteria	1. ~~Are~~ willing and able to provide informed consent for participation in the study	1. Subject / parent is willing and able to provide informed consent for participation in the study	Parent is included in all informed consent language for inclusion of pediatric subjects
	2. • ~~Cohorts 1-4 (5 and 6, if applicable)~~: ≥18 years of age • ~~MTD cohort:~~ ≥10 years of age	2. • Part I: ≥18 years of age • Part II: ≥10 years of age	
	5. Cohort 4~~ (5 and 6, if applicable) and MTD cohort~~:	5. Cohort 4-6 and Part II:	
8.2 Exclusion Criteria	2. Are unwilling to use barrier contraception methods, for a period of 3 months following treatment with AAV8-RPGR	2. Are unwilling to use barrier contraception methods (if applicable), for a period of 3 months following treatment with AAV8-RPGR	
8.3 Subject Withdrawal Criteria	For subjects who withdraw consent, data will be collected through their last available study visit.	For subjects who withdraw consent/assent, data will be collected through their last available study visit.	Assent is included in all informed consent language for inclusion of pediatric subjects
9.1 Treatments Administered	Subjects will receive either a dose of 5×10^9 gp (Cohort 1), 1×10^{10} gp (Cohort 2), 5×10^{10} gp (Cohort 3) or 1×10^{11} gp (Cohort 4) AAV8-RPGR. ~~In applicable, additional cohorts 5 (2.5×10^{11} gp) and 6 (5×10^{11} gp) may be added depending on observed toxicity~~ (see Section 7.3 for details).	Subjects will receive an AAV8-RPGR dose of 5×10^9 gp (Cohort 1), 1×10^{10} gp (Cohort 2), 5×10^{10} gp (Cohort 3), 1×10^{11} gp (Cohort 4), 2.5×10^{11} gp (Cohort 5), or 5×10^{11} gp (Cohort 6). (see Section 7.3 for details).	
9.5 Randomisation	~~Not applicable for this study.~~	The dose-escalation portion of this study is not randomized. In Part II, after the study eye is assigned, subjects will be randomised in a 2:1 ratio to receive either AAV8-RPGR MTD or a lower dose of AAV8-RPGR, three dose-	

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
		levels from MTD (e.g., low dose = 5 x 10^{10} gp if MTD = 5 x 10^{11} gp) for the active-control cohort. Randomisation will be generated using a validated system that automates the random assignment of treatment groups to randomisation numbers. Once a subject is deemed eligible, the investigative site (or authorised designee) will access the system, and the subject will be randomised using a standard blocked randomisation. The randomisation number will include the centre number and subject number.	
9.6 Study Masking	~~Due to the invasiveness of the vitrectomy and sub-retinal injection, both the investigator and the subject will be unmasked to the study procedure.~~	Part I of the study is open-label. Part II is double masked (subject, surgeon, investigator/site team, sponsor will be masked to the assigned dose, and open-label with respect to the treatment administration).	
10.1 Visit 1 (Screening /Baseline Visit)	The subject will sign and date one 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.	The subject or parent will sign and date one 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 or parent. Where applicable, an assent form will be completed by the subject.	Parent is included in all informed consent language for inclusion of pediatric subjects
	Screening procedures will consist of the following:	Screening assessments will be considered baseline measurements and will consist of the following:	

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
		• Randomisation** **Randomisation for Part II only.	
	Subjects who meet all of the inclusion criteria and none of the exclusion criteria will ~~be enrolled into the study and assigned a subject number.~~	Subjects who meet all of the inclusion criteria and none of the exclusion criteria will have a study eye assigned and be enrolled into the study. In Part II, subjects will be then randomised to the AAV8-RPGR treatment groups (MTD cohort or active-control cohort), and will remain masked to the treatment dose. See Section 9.5 for details on randomisation and assignment of subject numbers.	
	~~LLVA~~		Low luminance visual acuity testing removed; no rationale on first post-op day
10.3 Visit 3 (Day 1 Post-Operative Visit)	Subjects will be reminded of the requirement to use barrier contraception for a period of 3 months from the time of treatment.	Where applicable, subjects will be reminded of the requirement to use barrier contraception for a period of 3 months from the time of treatment.	Inclusion of pediatric patients
	~~LLVA~~		Low luminance visual acuity testing removed; no rationale on first post-op day
Visit 4 (Day 7 Post-Operative Visit)	~~Blood pressure and pulse~~		No rationale for vitals on month 1, increases study burden on site
Visit 5 (Month 1)	~~Blood pressure and pulse~~		No rationale for vitals on month 1, increases study burden on site
Visit 9 (Year 1)	**10.10 Visits 10 (Month 18 ± 14 Days) ~~and 11 (Year 2 ± 14 Days, End of Study Visit)~~**	10.10 Visit 10 (Month 18 ± 14 Days)	The procedures are no longer identical on these two visits so they have been separated

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
10.10 Visits 10 (Month 18 ± 14 Days) and 11 (Year 2 ± 14 Days, End of Study Visit)	~~Visual fields~~ ~~Contrast sensitivity test~~		These procedures have been removed from the visit to lessen the burden on the site.
10.10 Visit 10 (Month 18)	• ETDRS BCVA~~*~~ • LLVA~~*~~ *~~Assessments collected in triplicate at Visit 11 (Year 2). To facilitate triplicate testing, the visit should be conducted over 2 days. It is recommended to measure BCVA and LLVA twice on the first day and once on the second day (prior to pupil dilation). All 3 BCVA and all 3 LLVA values must be recorded in the eCRF. LLVA should be conducted immediately after each BCVA assessment.~~	• ETDRS BCVA LLVA	These assessments are no longer done in triplicate on this visit to lessen the burden on the site.
		10.11 Visit 11 (Year 2 ± 14 Days, End of Study Visit) At Visits 11 the following ocular assessments will be performed: • Full ophthalmic examination, including indirect ophthalmoscopy, a slit-lamp examination, IOP, anterior chamber and vitreous inflammation grading and LOCS III cataract grading • ETDRS BCVA* • SD-OCT • LLVA* • Fundus autofluorescence • Microperimetry • Fundus photography • Visual Fields • Contrast sensitivity test	

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
		• Colour vision test • Speed reading test • FST • AE/SAE monitoring • Concomitant medication review • * Assessments collected in triplicate. To facilitate triplicate testing, the visit should be conducted over 2 days. It is recommended to measure BCVA and LLVA twice on the first day and once on the second day (prior to pupil dilation). All 3 BCVA and all 3 LLVA values must be recorded in the eCRF. LLVA should be conducted immediately after each BCVA assessment.	
10.11 Visit 11 (Year 2)	~~• Blood pressure and pulse~~ ~~Collection of safety blood samples (haematology and clinical chemistry)~~		These have been removed as there was no rationale and lessened burden on the site.
10.12 Early Termination Visit	~~Details of the statistical analyses will be described separately in the Statistical Analysis Plan.~~ ~~• Summaries will be generated by dose and overall.~~	For full details of the statistical analyses, please refer to the XIRIUS Statistical Analysis Plan (SAP).	
12.2.2 Recording of Adverse Events	SAEs will be collected from the time the subject provides written informed consent through Visit 11 (or ET Visit or Unscheduled Visits, if applicable).	SAEs will be collected from the time the subject or parent (where applicable) provides written informed consent through Visit 11 (or ET Visit or Unscheduled Visits, if applicable).	Parent is included in all informed consent language for inclusion of pediatric subjects

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
12.2.3 Follow-up of Adverse Events	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 medication 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.	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 or parent (where applicable) withdraws consent or is lost to follow-up. All SAEs, regardless of attribution to study medication or the surgical procedure, should be followed-up until the event has resolved, subsided, stabilised or the subject or parent (where applicable) withdraws consent or is lost to follow-up.	Parent is included in all informed consent language for inclusion of pediatric subjects
13.0 Statistical Considerations	Due to the nature of the study design, no formal sample size computation was performed.	Due to the nature of the study design, no formal sample size computation was performed. A sample size of 30 subjects at the MTD dose ensures that events with an incidence ≥10% will be identified with a 95% probability.	Added sample size calculation
13.1 Sample Size	(Study Eyes versus ~~Control Eyes~~)	(Study Eyes versus Fellow Eyes).	Clarified the definition of the untreated eye.
13.4 Descriptive Statistics	No formal statistical comparison will be performed ~~(no p-value will be computed).~~		Clarification
	Continuous data will be summarised over time using mean, standard deviation, median, minimum and maximum.	Continuous data will be summarised over time using mean, and its 95% CI, standard deviation, median, minimum and maximum. 95% CIs will be 2-sided. Summaries will be generated by dose and overall, in Part I and, by group (MTD dose and low-dose) in Part II.	Clarification
	Demographics ~~will be summarised by subject and;~~	Demographics and baseline ocular characteristics will be summarised for the safety	Clarification

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
	baseline ocular characteristics will be summarised ~~by eye.~~	analysis set and the full analysis set.	
13.5 Demographics and Baseline Characteristics	(Study Eyes versus ~~Control Eyes~~)	(Study Eyes versus Fellow Eyes).	Clarification
13.6 Safety Analysis		No formal statistical testing will be performed for safety analyses. Safety analyses will be performed on the Safety Analysis Set.	Clarification
13.7.1 Alpha Adjustment	Alpha adjustment is not applicable in this study ~~as no formal comparison will be performed.~~	Alpha adjustment is not applicable in this exploratory Phase 1/2 study as no formal comparison will be performed.	Clarification
13.7 Interim Analysis		[redacted]	Interim analysis added.
13.8 Interim Analysis	Subjects must personally sign and date the latest Independent Ethics Committee (IEC)/Institutional Review Board (IRB) approved version of the informed consent form before any study-specific procedures are performed.	Subjects or parent (where applicable) must personally sign and date the latest IEC/ IRB approved version of the informed consent form before any study-specific procedures are performed. Where applicable a subject assent form will be obtained.	Assent is included in all informed consent language for inclusion of pediatric subjects
14.1 Informed Consent	Written and verbal versions of the subject information and informed consent will be presented to the subjects detailing no less than	Written and verbal versions of the subject information and informed consent will be presented to the subject or parents (where applicable)	Parent is included in all informed consent language for inclusion of pediatric subjects
	The subject will be allowed as much time as needed to consider the information and the opportunity to question the investigator,	The subject or parent (where applicable) will be allowed as much time as needed to consider the information and the opportunity to question the investigator,	Parent is included in all informed consent language for inclusion of pediatric subjects
	Written informed consent will then be obtained by means of	Written informed consent will then be obtained by means of	Parent is included in all informed consent

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text** **(Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text** **(Added Text Shown as Red)**	**Rationale**
	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.	subject or parent (where applicable) dated signature and dated signature of the person who presented and obtained the informed consent. Where applicable, a subject assent form will be obtained. The person who obtained the consent/assent 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 or parent.	language for inclusion of pediatric subjects
	The protocol, informed consent form,	The protocol, informed consent/assent form,	Assent is included in all informed consent language for inclusion of pediatric subjects
14.2 Ethical/Regulatory Review	The study 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)~~.	The study will be conducted in accordance with the relevant articles of the Declaration of Helsinki.	Unnecessary
14.3 Regulatory Considerations	Informed consent	Informed consent/assent	Assent is included in all informed consent language for inclusion of pediatric subjects
Table 3	Blood pressure and pulse		Removed from Visit 5, 9, ET; These have been removed as there was no rationale and lessened burden on the site.
	Collection of safety blood samples		Removed from Visit ET; These have been removed as there was no rationale and

SUMMARY OF CHANGES PROTOCOL NSR-RPGR-01 Version 6.0, 18 MAY 2018			
Section, Page	**Previous Text (Deleted Text Shown by ~~Strikethrough~~)**	**Revised Text (Added Text Shown as Red)**	**Rationale**
			lessened burden on the site.
	BCVA and LLVA		Not in triplicate at Visit 10; there was no rationale and lessened burden on the site.
		Corticosteroid compliance review	Added at Visits 2, 3, 4, 5
		Randomisation[n] [n]Part II only	Added at Visit 1
	~~LLVA~~		Removed from Visits 3, 4; there was no rationale and lessened burden on the site.

SUMMARY OF CHANGES FOR CLINICAL STUDY PROTOCOL NSR-RPGR-01

A Dose Escalation, Phase 1/2 Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

XIRIUS STUDY

INDICATION: X-linked retinitis pigmentosa

STUDY PHASE: 1/2

EUDRACT NUMBER: 2016-003852-60

SPONSOR: NightstaRx Ltd
Wellcome Gibbs Building, 215 Euston Road
London NW1 2BE, UK
Telephone: +44 (0) 207 611 2077

SUMMARY OF CHANGES: Protocol Version 5.0
16 Jan 2018

CONFIDENTIALITY STATEMENT

SUMMARY OF CHANGES SPONSOR APPROVAL PAGE

Clinical Study Protocol Number:	NSR-RPGR-01
Protocol Title:	A Dose Escalation, Phase 1/2 Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)
Summary of changes for Protocol:	Version 5.0 16 Jan 2018

Approved By:

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

Signed: [redacted] Date: 16 Jan 2018

[redacted] MD

Amendment 4.0, 16 Jan 2018
Amendment: Updated inclusion criterion #1 and exclusion criterion #2
Rationale: To clarify and allow the consent of male subjects ≥10 years who would require a parental permission and subject assent (if applicable). To clarify exclusion criteria # 2 for male subjects ≥10 years where applicable.
Sections: • Protocol synopsis • 7.1 Overall study design • 8.1 Inclusion criteria • 8.2 Exclusion criteria • 8.3 Subject withdrawal criteria • 10.1 Visit 1 • 10.3 Visit 3 • 12.2.2 Recording of adverse events • 14.1 Informed consent • 14.2 Ethical/ regulatory review
Amendment: Other minor changes throughout the document including removing the following study procedures at visit 5, visit 11 and ET: • Blood pressure and pulse • Collection of safety blood samples
Rationale: To improve clarity or correct typographical errors.
Sections: Throughout protocol.

SUMMARY OF CHANGES FOR CLINICAL STUDY PROTOCOL NSR-RPGR-01

A Dose Escalation, Phase 1/2 Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

XIRIUS STUDY

INDICATION: X-linked retinitis pigmentosa

STUDY PHASE: 1/2

EUDRACT NUMBER: 2016-003852-60

SPONSOR: NightstaRx Ltd
Wellcome Gibbs Building, 215 Euston Road
London NW1 2BE, UK
Telephone: +44 (0) 207 611 2077

SUMMARY OF CHANGES: Protocol Version 4.0
15 Dec 2017

CONFIDENTIALITY STATEMENT

SUMMARY OF CHANGES SPONSOR APPROVAL PAGE

Clinical Study Protocol Number: NSR-RPGR-01

Protocol Title: A Dose Escalation, Phase 1/2 Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

Summary of changes for Protocol: Version 4.0

15 Dec 2017

Approved By:

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

Signed: [redacted] Date: 15 DEC 2017

[redacted], MD
[redacted]

Amendment 3.0, 15 Dec 2017
Amendment: Updated study design to confirm assessment of Cohort 4 (1 x 10^{11} gp) as standard, and potential Cohorts 5 (2.5 x 10^{11} gp) and 6 (5 x 10^{11} gp).
Rationale: To allow wider dose exploration, if appropriate.
Sections: • Protocol synopsis • 7.3 Dose escalation scheme
Amendment: Updated study design to include additional Visits 10 (Month 18) and 11 (Year 2).
Rationale: To allow longer duration of post-treatment follow-up (24 months in total), in line with feedback received from regulatory agencies.
Sections: • Protocol synopsis • 7.1 Overall study design • 10.10 Visit 10 and 11 • 17.1 Schedule of study procedures
Amendment: Updated Maximum Tolerated Dose (MTD) cohort eligibility criteria to include patients ≥10 years of age.
Rationale: To allow inclusion of a wider study population in the MTD cohort, after safety and tolerability is first demonstrated in adults, considering X-linked Retinitis Pigmentosa is associated with early-onset of symptoms in childhood, and some children as young as 10 years of age can have significant disease affecting the central macula (i.e. potential candidates for gene therapy).

Sections:

- Protocol synopsis
- 7.5 Discussion of study design and dose selection
- 8.1 Inclusion criteria

Amendment:

Updated eligibility criteria to specify that ellipsoid zone at screening must be within the borders of the SD-OCT scan.

Rationale:

To ensure that participating patients have active disease within the macular region. Otherwise, the central reading centre is unable to adequately grade the SD-OCT images, which would negatively impact secondary study endpoints.

Sections:

- Protocol synopsis
- 8.1 Inclusion criteria

Amendment:

Updated Dose Limiting Toxicity (DLT) definition to exclude events related to the surgical procedure.

Rationale:

From the literature, it is known that the vitrectomy almost always leads to a transient, but significant, drop in visual acuity immediately post-operatively. This can be confused with a DLT event and therefore clarification is provided in the protocol to ensure only genuine cases of DLTs are identified and reported.

Sections:

- Protocol synopsis
- 7.2 Dose limiting toxicity

Amendment:

Removed several study assessments from specific visits, e.g.:

- Blood pressure & pulse (Visits 5, 9 and ET)
- Viral shedding (Visit 6)

• FAF (Visit 4) • Triplicate MP and VF (Visits 9 and ET)
Rationale: Following feedback from the central reading center, and several study sites, the utility of above assessment at these specific time-points is considered low.
Sections: • 10.4-10.6, 10.9 and 10.11 (Visits 4-6, 9 and ET) • 17.1 Schedule of study procedures
Amendment: Other minor changes throughout document.
Rationale: To improve clarity or correct typographical errors.
Sections: Throughout protocol.

SUMMARY OF CHANGES FOR CLINICAL STUDY PROTOCOL NSR-RPGR-01

A Dose Escalation, Phase 1/2 Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

XIRIUS STUDY

INDICATION: X-linked retinitis pigmentosa

STUDY PHASE: 1/2

EUDRACT NUMBER: 2016-003852-60

SPONSOR: NightstaRx Ltd
Wellcome Gibbs Building, 215 Euston Road
London NW1 2BE, UK
Telephone: +44 (0) 207 611 2077

SUMMARY OF CHANGES: Protocol Version 3.0 (amendment 2.0)
26 May 2017

CONFIDENTIALITY STATEMENT

SUMMARY OF CHANGES SPONSOR APPROVAL PAGE

Clinical Study Protocol Number:	NSR-RPGR-01
Protocol Title:	A Dose Escalation, Phase 1/2 Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)
Summary of changes for Protocol:	Version 3.0 (amendment 2.0) 26 May 2017

Approved By:

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

Signed: [redacted] Date: 2nd JUNE 2017

[redacted], MD

[redacted]

Amendment 2.0, 26 May 2017
Amendment: Update inclusion criterion for BCVA in MTD cohort.
Rationale: To allow inclusion of a wider study population into the MTD cohort.
Sections: • Protocol synopsis • 7.3 Dose escalation scheme • 8.1 Inclusion criteria
Amendment: Low luminance visual acuity assessment and full field stimulus threshold have been added as additional assessments. LLVA will be conducted at the same timepoints as EDTRS BCVA, FST will be conducted at baseline/screening, month 6, year 1 and Early termination visit
Rationale: LLVA and FST tests have a potential to provide additional data to measure changes in disease progression over time.
Sections: **LLVA** • Protocol Synopsis • 7.1 Overall Study Design • 10.1 Visit 1 (Screening/Baseline Visit) • 10.3 Visit 3 • 10.4 Visit 4 • 10.5 Visit 5 • 10.6 Visit 6 • 10.7 Visit 7 • 10.8 Visit 8 • 10.9 Visit 9 • 10.10 Early Termination visit • 11.7 Low Luminance Visual Acuity

- 17.1 Schedule of Procedures

FST

- Protocol synopsis
- 7.1 Overall study design
- 10.1 Visit 1 (Screening/Baseline Visit)
- 10.7 Visit 7
- 10.9 Visit 9
- 10.10 Early Termination Visit
- 11.8 Full Field Stimulus Threshold Test
- 17.1 Schedule of Procedures

Amendment:
Remove microperimetry assessment at visit 4.

Rationale:
The utility of microperimetry at visit 4 (Day 7 post-op) is expected to be low.

Sections:

- 10.4 Visit 4
- 17.1 Schedule of Procedures

Amendment:
Update schedule of procedures to include triplicate testing at screening/baseline, year 1 and early termination visit for: EDTRS BCVA, LLVA, microperimetry and visual fields.

Rationale:
Following feedback from the central reading center, and several study sites, triplicates will improve data quality by mitigating potential learning effects and providing a measure of test-retest variability.

Sections:

- 10.1 Visit 1 (Screening/Baseline Visit)
- 10.9 Visit 9
- 10.10 Early Termination Visit
- 17.1 Schedule of Procedures

Amendment:
Adding corticosteroid compliance review

Rationale: To clarify that corticosteroid compliance will be monitored.
Sections: • 10.2 Visit 2 • 10.3 Visit 3 • 10.4 Visit 4 • 10.5 Visit 5
Amendment: Screening/baseline visit timing changed, from within 4 weeks of Visit 2 (±2 weeks), to within 8 weeks of Visit 2 (±2 weeks).
Rationale: To provide more flexibility in scheduling subsequent visits.
Sections: • 10.1 Visit 1 (Screening/Baseline Visit) • 17.1 Schedule of Procedures
Amendment: Other minor changes throughout document.
Rationale: To improve clarity or correct typographical errors.
Sections: Throughout protocol.

SUMMARY OF CHANGES FOR CLINICAL STUDY PROTOCOL NSR-RPGR-01

A Dose Escalation, Phase 1/2 Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)

XIRIUS STUDY

INDICATION: X-linked retinitis pigmentosa

STUDY PHASE: 1/2

EUDRACT NUMBER: 2016-003852-60

SPONSOR: NightstaRx Ltd
Wellcome Gibbs Building, 215 Euston Road
London NW1 2BE, UK
Telephone: +44 (0) 207 611 2077

SUMMARY OF CHANGES: Protocol Version 2.0 (amendment 1.0)
01 Dec 2016

CONFIDENTIALITY STATEMENT

SUMMARY OF CHANGES SPONSOR APPROVAL PAGE

Clinical Study Protocol Number:	NSR-RPGR-01
Protocol Title:	A Dose Escalation, Phase 1/2 Clinical Trial of Retinal Gene Therapy for X-linked Retinitis Pigmentosa Using an Adeno-Associated Viral Vector (AAV8) Encoding Retinitis Pigmentosa GTPase Regulator (RPGR)
Summary of changes for Protocol:	Version 2.0 (amendment 1.0) 01 Dec 2016

Approved By:

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

Signed: [redacted] Date: 1st Dec. 2016.

[redacted], MD
[redacted]

Amendment 1.0, 01 Dec 2016
Amendment: Update exclusion criterion 2 to include time frame (3 months), within which subjects are to be compliant with the use of barrier contraception.
Rationale To clarify both the time frame (3 months) and that the requirement applies from the time subjects receive AAV8-RPGR.
Sections: • 2. Protocol Synopsis • 8.2. Exclusion Criteria • 10.1. Visit 1 (Screening/Baseline Visit) • 10.3. Visit 3 (Day 1 Post-Operative Visit)