THOR - Tübingen Choroideremia gene therapy trial
open label Phase 2 clinical trial using an adeno-associates viral vector (AAV2) encoding Rab-escort protein 1 (REP1)

Version 2.0

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## Amendment History

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<td>2</td>
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<td>Definition of responder (ETDRS) was changed to &gt;5 on p10, p17 and 10.4 on p47</td>
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<td>4</td>
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<td>9.11.2015</td>
<td>Both eyes will be randomized to either study or control eye p10, p16, and 5.2 on page 26</td>
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<td>5</td>
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<td>In section Treatment 6.1 page 26 the advice “For further details see SOP vector administration” was added</td>
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<td>Annex B and C were deleted because an updated IB is available with more recent information</td>
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<td>More details on expected adverse reactions were added p29/30</td>
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<td>8.2.14.1 Immunoassay methods were described more precisely</td>
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II Abbreviations

µl  microliter
µm  micrometer
AAV2  Adeno associated virus
AE  adverse event
AIS  electronical patient file
AR  adverse reaction
BGH  Bundesgerichtshof
BSS  balanced salt solution
CHM  Choroideremie
cm  centimeter
CRF  case report form
CRO  clinical research organisation
CT  Cycle threshold
d  day
DMC  data monitoring committee
DNA  desoxyribo nucleic acid
e.g.  exempli gratia
EC  ethics committee
ERG  electroretinogram
ETDRS  early treatment diabetic retinopathy study
GFP  green fluorescent protein
GMP  good manufacturing pratice
gp  genome particle
GTAC  gene therapy advisory committee
i.e.  id est
ICH-GCP  international conference of harmonization-good clinical practice
IMP  investigational medical product
ITR  inverted terminal repeat
ITT  intent to treat
kg  kilogram
mfERG  multifocal electroretinography
mg  milligram
MHRA  Medicines and Healthcare products Regulatory Agency
ml  milliliter
MMP1  microperimetry
mRNA  messenger ribonucleic acid
N  number of patients
NHS  National Health Service
OCT  optical coherence tomography
PCR  polymerase chain reaction
PEI  Paul-Ehrlich-Institute
PI  principal investigator
PP per protocol
qPCR quantitative polymerase chain reaction
rAAV2.REP1 AAV with DNA for the gene REP1
REP1 Rab escort protein 1
RPE65 gene
SAE serious adverse event
SAR serious adverse reaction
SLO
SLO scanning laser ophthalmoscope
SOP standard operating protocol
STZ Steinbeis-Transferzentrum
SUSAR suspected unexpected serious adverse reaction
UKT University Hospitals Tuebingen
vgp virus genome particle
w week
WPRE posttranscriptional regulatory element of woodchuck hepatitis virus
III Roles in the Study

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**Funder**
Tistou and Charlotte Kerstan Foundation

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IV Synopsis

Study name: THOR - Tübingen Choroideremia gene therapy trial open label Phase 2 clinical trial using an adeno-associated viral vector (AAV2) encoding Rab-escort protein 1 (REP1)

Phase: Phase II

Indication: Adult males with a clinical phenotype of choroideremia and a confirmed molecular diagnosis of a null mutation in the gene encoding REP1

Aim of study: To assess the anatomical and functional outcomes, as well as the safety of a single subretinal injection of rAAV2.REP1 in subjects with genetically confirmed choroideremia for up to 24 months.

Primary Endpoint: Change from baseline in best corrected visual acuity in treated eye, compared to untreated control eye

Study design: Open label monocenter study

Study population: 6 male adults affected by choroideremia

Inclusion Criteria
1. Participant is willing and able to give informed consent for participation in the study.
2. Male aged 18 years or above.
3. Genetically confirmed diagnosis of choroideremia. Patients without a confirmed mutation in the CHM gene, but who have the clinical phenotype typical of choroideremia can only be enrolled if they meet all the following three criteria: (i) family history consistent with X-linked inheritance, (ii) absent REP1 protein on Western blot of a blood sample and, (iii) normal RPE65 gene on sequencing.
4. Active disease visible clinically within the macula region
5. Best-corrected visual acuity equal to or worse than 6/9 (20/32; Decimal 0.63; LogMAR 0.2) but better than or equal to 6/60 (20/200; Decimal 0.1; LogMAR 1.0) in the study eye.

Exclusion Criteria
1. Female and child participants (under the age of 18)
2. Participants with a history of amblyopia in the study eye
3. Men unwilling to use barrier contraception methods, if relevant
4. Absence of quantifiable visual function in the fellow eye or other ocular morbidity which might confound use of the fellow eye as a long-term control.
5. Any other significant ocular and non-ocular disease/disorder or retinal surgery which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the results of the study, or the participant’s ability to participate in the study. This would include not taking or
having a contraindication to oral prednisolone, such as a history of gastric ulcer or significant side effects.

6. Participants who have participated in another research study involving an investigational product in the past 12 weeks, or having had gene or cellular therapy at any time prior to this study.

7. Patients with amblyopic eyes should be excluded in general, since the evaluation of the primary endpoint presupposes the ability to fixate both eyes

8. Prior intraocular surgery within six months

9. Intolerance to local anesthesia and/or contraindication to IVT surgery (anemia Hb<8g/dl, severe cardiovascular disease, severe coagulopathy, etc.)

10. High fever or high fever disease, patients with a history of autoimmune conditions/other systemic diseases that may have ocular manifestations (e.g. sarcoidosis) or neurodegenerative conditions (e.g. multiple sclerosis, neuromyelitis optica, Parkinson’s disease)

11. Patients suffering from other genetic mutations leading to pathological retinal conditions

12. Patients treated by oral corticoids within 14 days prior inclusion at the study entry

Patient number: 6

Treatment: Each participant will receive a single treatment of the rAAV2.REP1 vector (0.1ml containing $10^{11}$ AAV2 genome particles) administered by subretinal injection during a vitrectomy operation. No placebo will be used. In order to minimize selection bias both eyes are randomized to either treatment or control.

Main criteria: Primary endpoint: Change from baseline in best corrected visual acuity in treated eye, compared to untreated control eye up to 24 months after vector administration

Secondary endpoints: Absence of vector related adverse reactions 24 months after vector administration. Demonstration of improved retinal anatomy and/or visual function other than best corrected visual acuity in treated eye compared to the untreated control eye 24 months after vector administration.

Statistical methods:

Summary statistics will be presented for both eyes (Treated Eye versus Control Eye groups). No formal statistical comparison will be performed (no p-value will be computed). For categorical/binary data, the number and proportion of patients in each category will be presented with its 95% Confidence Interval (CI). For continuous data, mean (and its 95% CI) and Standard Deviation (SD) will be presented.

The primary outcome measure will be the proportion of patients with a relative change from baseline of > 5 in ETDRS letters (treated vs. untreated eye, change from BL). At each time point, the change from baseline in ETDRS letters will be computed for each eye. The mean change from baseline in ETDRS letters will be presented for both the treated eye and the control eye groups.
At each time point, the change from baseline and the percentage change from baseline in the area of autofluorescence will be computed for each eye and their mean will be presented for both the treated eye and the control eye groups.

With regards to microperimetry, at each time point, the change from baseline in mean sensitivity will be computed for each eye. The mean change from baseline in mean sensitivity will be presented for both the treated eye and the control eye groups.

Adverse events will be listed.

Other Investigator Sponsored Studies are expected to be run with a similar protocol for the same indication and with the same intervention. A meta-analysis on the Investigator Sponsored studies is planned. A separate Statistical Analysis Plan describing the details of the meta-analysis will be developed.

**Timetable:**
Planned trial period: 24 month
Follow-up duration: 36 month

The end of trial is the date on which the last treated patient completes the tests of their planned close-out visit.

**Study process:** Flow-chart see next page
### Assessments/Procedures

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All procedures performed in both eyes.

a Screening visit must be performed within 2 weeks of Visit 2.

b Urea, electrolytes, liver function tests

c Viral shedding assays include blood, tears, saliva, urine
e  Perform blood CRP, Total IgM, Total IgG
f  Perform ELISA & ELISPOT
g  Full ophthalmic exam to include: intraocular pressure, slit lamp examination, lens opacity and dilated ophthalmoscopy examination. The same slit lamp machine and lighting conditions should be used across study visits for a given subject.
h  Pelli Robson chart will be used for Contrast Sensitivity
i  Colour vision testing will be performed using Roth 28 test
j  Stereo photos for Fields 1, 2, 3.
k  Early termination visit to be performed if a subject discontinues any time following the administration of the rAAV2.REP1 injection.
l  BCVA, Full Ophthalmic Exam, SD-OCT, Autofluorescence, Microperimetry, Concomitant Medication and AE/SAE Monitoring as minimum if clinically required
V Sponsor and Investigator Agreement

Sponsor of this clinical trial is the University Hospital Tübingen, Geissweg 3, 72076 Tübingen.

The sponsor is represented by the Principal Investigator or his deputy and confirms that this protocol entitled “THOR - Tübingen Choroideremia gene therapy trial open label Phase 2 clinical trial using an adeno-associated viral vector (AAV2) encoding Rab-escort protein 1 (REP1)” Version 1.0 (EudraCT 2014-005004-21) has been carefully read and fully understood, and there is agreement to comply with the conduct and terms of the study specified herein in compliance with Good Clinical Practice and all other relevant regulatory requirements.

Principal Investigator (PI)____________________________________

Date _____________________________________________________

Signature__________________________________________________

Deputy Principal Investigator _________________________________

Date _____________________________________________________

Signature__________________________________________________
VI Synopsis in German / Deutsche Prüfplan-Zusammenfassung

Titel:
THOR – eine offene Phase II Choroideremie Gentherapiestudie in Tübingen mit einem adeno-assoziierten viralen Vektor (AAV2), der das Rab-escort Protein 1 (REP1) verschlüsselt.

Phase: II

Indikation: Erwachsene Männer mit einem klinischen Phänotyp der Choroideremie und einer bestätigten molekularen Diagnose einer pathogenetischen Mutation des REP1-codierenden Gens.

Studienziel: Ziel der Studie ist es, sowohl die anatomischen und funktionellen Ergebnisse, als auch die Sicherheit einer einzelnen subretinalen Injektion des rAAV2.REP1 in Patienten mit genetisch bestätigter Choroideremie für 24 Monate zu beurteilen.

Primärer Endpunkt: Unterschied der bestkorrigierten Sehschärfe (bestimmt bei der Baselineuntersuchung) im behandelten Auge, verglichen mit dem unbehandelten Kontrollauge.

Studien Design: Offene, monozentrische Studie

Studienpopulation: 6 männliche Erwachsene mit Choroideremie

Einschlusskriterien:
1. Patient willens und fähig, seine Einverständniserklärung zur Studie zu geben
2. Männlich und 18 Jahre oder älter
3. Genetisch bestätigte Choroideremie-Diagnose. Patienten ohne bestätigte Mutation im CHM Gen, die aber den für Choroideremie typischen klinischen Phänotyp aufweisen, können nur eingeschlossen werden, wenn die folgenden 3 Kriterien auf sie zutreffen: (i) Familiengeschichte vereinbar mit X-chromosomaler Vererbung (ii) nicht vorhandenes REP1 Protein im Western Blot einer Blutprobe und (iii) normales RPE65 bei der Sequenzierung.
4. Aktiver Krankheitsverlauf mit sichtbaren Veränderungen in der Makula-Region
5. Bestkorrigierte Sehschärfe, gleich oder schlechter als 6/9 (20/32; Decimal 0.63; LogMAR 0.2), aber besser als oder gleich 6/60 (20/200; Decimal 0.1; LogMAR 1.0) im Studienauge

 Ausschlusskriterien:
1. Frauen und Patienten unter 18 Jahre
2. Patienten mit vorangegangener Amblyopie im Studienauge
3. Männer, die - falls notwendig - nicht bereit sind Barriere-Verhütungsmethoden anzuwenden


6. Patienten, die in den letzten 12 Wochen an einer anderen Interventionsstudie teilgenommen haben, oder die vor dieser Studie eine Gen- oder zelluläre Therapie hatten.


8. Intraokulare Operation innerhalb der vergangenen 6 Monate

9. Unverträglichkeit lokaler Anästhetika und/oder Kontraindikation für eine intravitreale Operation (Anämie Hb<8g/dl, schwere vaskuläre Erkrankung, schwere Koagulopathie, etc.)

10. Hohes Fieber oder Krankheiten mit hohem Fieber, Patienten mit vorausgegangenen Autoimmunerkrankungen/anderen systemischen Erkrankungen, die sich im Auge manifestieren können (z.B. Sarkoidose) oder neurodegenerative Erkrankungen (z.B. Multiple Sklerose, Neuromyelitis optica, Parkinson)

11. Patienten, die unter genetischen Mutationen leiden, die zu pathologischen Veränderungen der Retina führen

12. Patienten, die innerhalb 14 Tage vor Einschluss in die Studie mit oralen Kortikoiden behandelt wurden

**Anzahl der Patienten:** 6

**Behandlung:** Jeder Proband erhält im Rahmen einer Vitrektomie eine einmalige subretinale Injektion des rAAV2.REP1 Vektors (0.1 ml rAAV2.REP1, die 10^{11} Genompartikel enthält). Es werden keine Placebos eingesetzt. Der AAV-Vektor wird wie ein Arzneimittel behandelt und jedem Studienteilnehmer unter genauer Dokumentation verabreicht. Um Voreingenommenheit auszuschließen, werden beide Augen randomisiert und der Behandlungs- bzw. Kontrollgruppe zugeteilt.

**Statistische Methoden:**

Für den Vergleich beider Augen werden zusammenfassende Statistiken herangezogen. (Gruppen behandelter Augen versus Kontrollaugen). Es wird kein formaler statistischer Vergleich durchgeführt (p-Werte). Für kartegoriale/binäre Daten werden die Anzahl und der Prozentsatz der Patienten jeder Kategorie sowie das 95% Konfidenzintervall angegeben (CI). Für kontinuierliche Daten, wird der Mittelwert (and sein 95% CI sowie die Standardabweichung (SD) angegeben.

Unerwünschte Ereignisse werden gelistet.

Es befinden sich weitere forschungsinitiierte Studien mit ähnlichen Studienprotokollen bei gleicher Indikation und Intervention in anderen Ländern (USA, Kanada, England) in Vorbereitung. Eine Metaanalyse über diese Studien ist geplant, für die derzeit ein Statistischer Analyseplan vorbereitet wird.

**Zeitplan:** Geplante Studiendauer: 24 Monate, Nachbeobachtungs-Phase: 36 Monate

Ende der Studie ist das Datum, an dem der letzte in der Studie behandelte Patient (Nr. 6) die Close-out Visite absolviert hat.
1 Introduction

1.1 Background

This is an investigator-initiated trial fully funded by the Tistou and Charlotte Kerstan Stiftung, Germany.

The adeno-associated viral vector (AAV2) encoding Rab-escort protein 1 (REP1) for this trial is provided by NightstaRx Ltd, UK, under the terms of a specific contract. Similar investigator-initiated trials are taking place in the UK (2 sites), Canada (1 site) and the United States of America (1 site) with the network of involved Principal Investigators collaborating, sharing study methodology and exchanging experience continuously.

Choroideremia

Choroideremia (CHM) is an untreatable retinal degeneration that begins in childhood with loss of night vision and gradually progresses to blindness by middle age. CHM is caused by loss of function of the gene encoding Rab escort protein 1 (REP1) which is located on the X-chromosome (Seabra et al., 1992; Seabra et al., 1993). Hence the disease has an X-linked recessive mode of inheritance; it affects approximately 1 in 50,000 people of European descent (Sankila et al., 1992; MacDonald et al., 2009).

Gene therapy with adeno-associated viral (AAV) vectors

Adeno-associated virus (AAV) is a parvovirus containing single stranded DNA. There are many different AAV subtypes, each with slightly different DNA sequences and capsid proteins. AAV serotype 2 (AAV2) is the vector proposed in this study. The wild-type AAV2 genome lacks many of the viral sequences necessary for packaging of viral particles and has evolved to become dependent on adenovirus for replication and spread from infected cells. For most of the time the AAV2 genome must remain dormant in the host cell, which may have helped it to evolve to remain undetected by the eukaryotic immune system, although wild-type AAV2 antibodies can be detected in about 30% of humans (Mingozzi and High, 2007). Over time, the wild-type AAV genome may become integrated into chromosome 19.

The non-immunogenic features of AAV2 make it ideal for gene therapy and over 500 people have been treated with it so far for a variety of conditions outside the eye. The principle is to remove the wild-type AAV2 gene and replace it with a specific therapeutic transgene before using this recombinant vector to deliver the therapeutic gene to diseased cells. The total size of the AAV2 genome is 4,700 base pairs and must include the inverted terminal repeat (ITR) sequences, which remain at either end of the transgene ((Lusby et al., 1980). Hence the main drawback of AAV2 is the relatively small size of gene it can carry. At 1,900 base pairs REP1 is fortunately well within this carrying capacity.

Gene therapy to the retina has advantages compared to other organs because the target area is small and much lower doses of vector can be applied by injection into the subretinal space, which is an enclosed natural anatomical space. The surgery is
relatively non-traumatic and can be performed under local anaesthetic. AAV vectors target neurons effectively and AAV2 can infect rods, cones and the retinal pigment epithelium after subretinal injection in non-human primates (Jacobson et al., 2006). These are the cells, which are primarily affected by the absence of REP1 in choroideremia, making the natural targeting of AAV2 ideal for this condition.

1.2 Rationale of the trial

Choroideremia is a disease that causes blindness, with no available treatment option. The initial results from 6 patients included in a Phase I/II study (Maclaren et al., 2014) show the investigational gene therapy medicinal product being well tolerated and although the study is not powered to show efficacy there are functional improvements in vision following retinal detachment and subfoveal injection of rAAV2.REP1 (0.6-1.0 x 10^{10} genome particles), performed under general anaesthesia. The initial results of this investigational retinal gene therapy trial are consistent with improved rod and cone function that overcome any negative effects of retinal detachment. These findings lend support to further assessment of rAAV2.REP1 gene therapy in the treatment of choroideremia.

Outline of proposed clinical trial

The purpose of the proposed study is to assess the safety and tolerability of the rRAAV2.REP1 vector, administered in 6 patients affected by choroideremia. There is now a considerable amount of data from clinical trials in 18 patients treated with subretinal injection of AAV2 in doses ranging from 10^{10} (Philadelphia - NCT00516477; Florida - NCT00481546) to 10^{12} (London - NCT00643747) AAV genome particles. There has so far been no SAR reported in any of these studies, confirming preclinical predictions that AAV is relatively non-immunogenic. We also know from another clinical trial for Parkinson’s disease, that a very similar AAV vector (with identical promoter and regulatory sequences and made by the same company) was used safely without SAR when infused directly into the subthalamic nucleus in 12 patients ((Kaplitt et al., 2007). In that study, AAV2 genome particle doses ranged from 5 x 10^{9} to 5 x 10^{10} (NCT00195143). None of the 12 patients developed antibody reactions to AAV2; including two patients with pre-existing titres to wild type AAV2 at baseline. Hence we can be reasonably confident, based on these observations, that the relatively small doses of AAV2 vector used in this trial are unlikely to trigger significant immune reactions, even if vector becomes disseminated from the eye into the brain.

The unknown factor in this proposed trial, however, is the REP1 protein and the potential detrimental effects this might have on cells if significantly over-expressed. There is no preclinical evidence that REP1 is toxic and it is present in virtually all nucleated cells in non-CHM patients. Nevertheless, in order to arrest the degeneration, we need to transduce retinal cells that are still functional. We plan to administer a vector dose of 0.1 ml containing 10^{11} genome particles. We have a highly efficient vector that has a strong promoter and optimised WPRE and BGH regulatory sequences (see Investigator Brochure). Furthermore, many of the retinal
cells in the central macula are functional and we can assume that they are not being subjected to a significant stress response. Translation of the therapeutic REP1 mRNA in these cells is therefore likely to be highly efficient.

**Previous gene therapy clinical trials**

Recently three landmark clinical trials (NCT00516477, NCT00481546 and NCT00643747) have shown safety and evidence of efficacy using adeno-associated viral (AAV) vectored gene therapy in a much rarer retinal degeneration caused by mutations in the gene encoding RPE65 (for full review, see MacLaren, 2009, and Investigator Brochure). The target cells, mode of delivery and AAV2 vector used in the previous clinical trials are very similar for CHM and the gene to be replaced (REP1) is almost the same size as RPE65. Several years have passed since the first retinal gene therapy trials started and there have been no reports so far of a serious adverse reaction (SAR) to the AAV2 vectors used. After the Phase 1/2 trial in CHM at Oxford has given encouraging results we plan an investigator-initiated trial with the same vector in Germany to give patients access to gene therapy within the setting of a Phase II clinical trial.

**1.3 Dose Selection**

Calculation of vector dose is estimated based on previous clinical trials using the AAV2 vector with a CBA promoter. The retinal gene therapy trials led by Maguire, Bainbridge and Hauswirth injected a total AAV dose (viral genomes) of $1.5 \times 10^{10}$, $1.5 \times 10^{11}$ and $6 \times 10^{10}$ respectively. None showed any detrimental effects, but the study by Maguire used surfactant, which prevented the adherence of AAV2 to the plastic in the injection system. In the presence of surfactant (0.001% Pluronic acid/ PF68; BASF, Ludwigshafen, Germany) virtually 100% of all vector entering the injection system passed through, but without surfactant 75% of vector was lost in the injection system, presumably as a result of internal binding of AAV2 to the plastic (Bennicelli et al., 2008). Data on final titre without surfactant in the other two studies is not available, but it seems reasonable to assume that the viral dose actually injected would have been less than the pre-injection titre. The second phase of the Maguire dose escalation study (Maguire et al., 2009) was completed without adverse reactions in three patients receiving a dose of $1.5 \times 10^{11}$. Hence the Maguire (Maguire et al., 2009) study probably represents the highest dose of AAV genomes that was definitely known to have been injected and shown to have been well tolerated. An initial dose of $1 \times 10^{10}$ genome particles was used for the first 6 patients in the University of Oxford Phase 1/2 study (Maclaren et al., 2014). The dose was well tolerated. In addition, 3 patients have been dosed at $1 \times 10^{11}$ genome particles, with no significant safety issues reported (see Investigators Brochure). The patients who had received the highest dose relative to the remaining target tissue showed the best evidence of efficacy. This confirms that $1 \times 10^{10}$ genome particles might be just below the therapeutic threshold and that $1 \times 10^{11}$ genome particles is a dose with a good safety profile in human subjects and has the highest chance of
showing therapeutic efficacy (Maclaren et al., 2014). A dose of $1 \times 10^{11}$ genome particles will therefore be used in this study.

1.4 Study Masking

This is an open label study, with no masking. However to minimise bias evaluation of the treated and non-treated eye, the following assessments will be conducted by an appropriately qualified masked observer: Fundus Autofluorescence, Microperimetry, SD-OCT, Visual Fields and Fundus Photography. A masked assessor not belonging to the core study team and not having any other tasks in the THOR study will assess BCVA as the primary endpoint of the trial.

2 Aim of study

2.1 Primary aim

The primary aim is to assess the anatomical and functional outcomes, as well as the safety of a single subretinal injection of rAAV2.REP1 in subjects with genetically confirmed choroideremia for up to 24 months. We predict that the REP1 transgene will be expressed efficiently if the retinal cells survive AAV2 transduction and this would be evidenced by sustained vision.

2.2 Secondary aims

Secondary study endpoints are, change from baseline in autofluorescence evaluation, microperimetry readings and other anatomic and functional outcomes (all in the study eye compared to control eye). Secondary endpoints also include safety assessments to be conducted throughout the study. The fellow eyes of these patients will be utilised as controls in this study and will receive no study treatment.

3 Study plan

3.1 Study design

3.2 Summary of Trial Design

This is an open label monocenter study involving a total of 6 male patients with a clinical phenotype of choroideremia and a confirmed molecular diagnosis of a null mutation in the gene encoding REP1. Molecular testing will take place in an approved Diagnostic Laboratory. The patients will receive treatment with the investigational gene therapy medicinal product: AAV2.CBA.REP1.WPRE.BGH (see Investigator Brochure). The patients will receive a subretinal injection of $10^{11}$ AAV2
genome particles, 3 patients at day 0 and 3 patients at day 7. Each patient will be followed up for 24 months after treatment to assess the primary and secondary endpoints of this study. Further follow-up will however continue annually up to five years as a follow-up to the study (continued annually within clinical routine) and data will continue to be analysed by members of the study group after this study is complete. At least one annual follow-up would be the routine in choroideremia patients as part of their normal ophthalmological care and in accordance with the guidelines for gene therapy.

Potential eligible patients will be identified in the hospital patient data base and by information via the German choroideremia patient organization. All images and scans will be pseudonymised and also kept electronically in the case report form (CRF) held by the Sponsor. Enrolment in the trial, retinal surgery and post-operative monitoring following treatment with vector will all be performed at the Sponsor site under direction of the Principal Investigator.

### 3.3 Study duration

- Planned trial period: 24 month
- Follow-up duration: 36 month

The end of trial is the date on which the last treated patient completes the tests of their planned close-out visit.
Figure 2: Schedule of injection intervals and Data monitoring committee (DMC) visits

DMC = Data monitoring committee
3.4 Participating Institutions

University Eye Hospital, Centre for Ophthalmology, University Hospitals Tübingen, Germany.

3.5 Number of enrolled patients

6 male adults affected by choroideremia.

4 Study Population

4.1 Characterization of population

All patients included in the study will be male, because the disease is linked via REP1 to the X chromosome. Although choroideremia carrier females may have visual impairment, the rate of progression is generally much slower and more variable than in affected males. Consequently it would be difficult to determine the secondary trial end point in terms of proven efficacy in female patients. The general principle will be to enrol patients during the most active stages of the disease when changes in retinal appearance are taking place in the posterior pole of the eye. This region can be subjected to detailed image analysis and analysis of functional testing. Hence patients with very early disease, which has minimal visible changes will not be enrolled. Effectively this will exclude children and accordingly the consent and information brochures are set up for adult subjects.

4.2 Inclusion Criteria

1. Participant is willing and able to give informed consent for participation in the study.
2. Male aged 18 years or above.
3. Genetically confirmed diagnosis of choroideremia. Patients without a confirmed mutation in the CHM gene, but who have the clinical phenotype typical of choroideremia can only be enrolled if they meet all the following three criteria: (i) family history consistent with X-linked inheritance, (ii) absent REP1 protein on Western blot of a blood sample and, (iii) normal RPE65 gene on sequencing.
4. Active disease visible clinically within the macula region
5. Best-corrected visual acuity equal to or worse than 6/9 (20/32; Decimal 0.63; LogMAR 0.2) but better than or equal to 6/60 (20/200; Decimal 0.1; LogMAR 1.0) in the study eye.
4.3 Exclusion Criteria

1. Female and child participants (under the age of 18)
2. Participants with a history of amblyopia in the study eye
3. Men unwilling to use barrier contraception methods, if relevant
4. Absence of quantifiable visual function in the fellow eye or other ocular morbidity which might confound use of the fellow eye as a long-term control.
5. Any other significant ocular and non-ocular disease/disorder or retinal surgery which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the results of the study, or the participant's ability to participate in the study. This would include not taking or having a contraindication to oral prednisolone, such as a history of gastric ulcer or significant side effects.
6. Participants who have participated in another research study involving an investigational product in the past 12 weeks, or having had gene or cellular therapy at any time prior to this study.
7. Patients with amblyopic eyes should be excluded in general, since the evaluation of the primary endpoint presupposes the ability to fixate both eyes
8. Prior intraocular surgery within six months
9. Intolerance to local anesthesia and/or contraindication to IVT surgery (anemia Hb<8g/dl, severe cardiovascular disease, severe coagulopathy, etc.)
10. High fever or high fever disease, patients with a history of autoimmune conditions/other systemic diseases that may have ocular manifestations (e.g. sarcoidosis) or neurodegenerative conditions (e.g. multiple sclerosis, neuromyelitis optica, Parkinson's disease)
11. Patients suffering from other genetic mutations leading to pathological retinal conditions
12. Patients treated by oral corticoids within 14 days prior inclusion at the study entry

5 Patient inclusion

5.1 Time of entry

Prior to enrolment patients will have confirmed sequencing of REP1 to confirm the molecular diagnosis in an accredited laboratory.

5.2 Mode of assignment of patients to treatment

Patients with a clinical diagnosis of choroideremia and a molecular diagnosis of a null mutation in the gene encoding REP1 will be invited to take part in the study. In addition to the baseline visual tests, those eligible and giving informed written
consent will also have their demographic information, medical history and concomitant medications recorded. They will also undergo a general examination to chart height, weight and temperature and measure resting pulse and blood pressure. Additional counselling about the implications of being in the study will be provided by a suitably qualified non-medical member of the research team. In order to minimize selection bias both eyes are randomized to either treatment or control.

6 Treatment

6.1 Treatment

Description of Study Treatment

Starting 1 day before treatment, immunosuppression by oral corticosteroids and topical steroids will be performed. Systemic steroids will be given orally at 1.0 mg/kg bodyweight for approximately 3 weeks starting at day -1 and then tapering off after day 19 at the discretion of the investigator (e.g 40/30/20/15/10mg). Moxifloxacin eye drops 0.5% are administered 4 times a day and dexamethasone gel 0.5% is administered 4 times a day for 21 days, starting at day -1.

0.1ml of rAAV2.REP1, containing 1 x 10^{11} genome particles, administered as a single subretinal injection. For details see SOP vector administration. There is no placebo or comparator product in this study. Since choroideremia affects both eyes in a generally symmetrical pattern, the fellow eye (which does not receive any study treatment) will be used as a comparator (Seitz et al., 2015).

Storage of Study Treatment

The vector will be stored frozen at -80 degrees Celsius (maximum temperature -60 degrees) at the Centre for Ophthalmology, University of Tübingen, thawed on the day of surgery and checked prior to anaesthetising the patient. The vector solution will be transferred to an injection syringe connected to a vitrectomy machine for precise and controlled intraocular fluid delivery and will remain ready for injection in this system for up to 3 hours.

Accountability of the Study Treatment

The rAAV2.REP1 vector will be supplied by NightStarX, UK, to the site in Tübingen and retrieved (unused medication and returned, used vials/packaging) at the end of the study. All movements of study medication between NightStarX and site will be documented as will be the specified usage of vials / secondary containers (cryoboxes) according to their labels on the accountability logs of the study unit STZ eyetrial at the Centre for Ophthalmology.
Post-trial treatment

Participants will continue normal care and receive eye drops or other medication as required (see also description of study treatment 6.1). Annual checks will continue at the local eye hospital as part of their routine care.

6.2 Definition of additional methods of treatment

Concomitant Medications and Procedures

Details of concomitant medication will be collected at the screening visit, and updated at every study visit (including any unscheduled visits). Throughout the study, Investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care. Any medication, (including anaesthetic and other surgical medications, but excluding study medication), taken during the study will be recorded in the patients’ medical records and CRF. Also medically relevant concomitant procedures will be subject to study documentation.

7 Main criteria for estimation of effectiveness and tolerance

7.1 Effectiveness

Primary Endpoint:
- Change from baseline in best corrected visual acuity in treated eye, compared to untreated control eye

Secondary Endpoints:
- Change from baseline in autofluorescence evaluation in treated eye, compared to untreated control eye
- Change from baseline in central visual field using microperimetry readings in treated eye, compared to untreated control eye
- Change from baseline in other anatomic and functional outcomes during the study in treated eyes, compared to untreated control eyes
- Safety evaluation during the study

7.2 Tolerance

Adverse Event (AE)

An AE or adverse experience is: Any untoward medical occurrence in a patient or clinical investigation participants administered a medicinal product, which does not
necessarily have to have a causal relationship with this treatment (the study medication).
An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the study medication, whether or not considered related to the study medication.

**Adverse Reaction (AR)**

ARs are all untoward and unintended responses to a medicinal product related to any dose. The phrase "responses to a medicinal product" means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.
All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.

**Severe Adverse Events**

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:
The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

**Serious Adverse Event (SAE)**

A serious adverse event is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening: the term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Results in other important medical events. Note: Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.
Serious Adverse Reaction (SAR)

An adverse event (expected or unexpected) that is both serious and, in the opinion of the reporting investigator, believed with reasonable probability to be due to one of the study treatments, based on the information provided.

Expected Serious Adverse Events/Reactions

Expected serious adverse events/reactions (SAE/SAR) are most likely to be related to (i) complications of retinal surgery, (ii) immune reactions to vector capsid, or (iii) immune reactions and/or toxic effects on retinal function caused by expression of human REP1 protein.

(i) Complications of retinal surgery: There are known complications of three port pars plana vitrectomy and subretinal surgery. These include retinal detachment, traumatic cataract, suprachoroidal and/or subretinal haemorrhage and endophthalmitis.

Retinal tears or entry site breaks
Periperal tears should be treated with laser and careful vitrectomy around the retinal tear to relieve any traction. Air or gas should not be used as this would displace the vector inferiorly away from the macula when the trial participant returns to the upright position. In cases of giant retinal tear, treatment should be confined to laser (or cryotherapy) only where possible, bearing in mind that the scar tissue formed as a result of chronic retinal degeneration in choroideremia will create a firm adherence that would limit progression of even large retinal tears compared with the normal course. Since most risk is in the foveal detachment, it is better to administer the vector wherever possible and this means not using air or gas. The chance of retinal tears progressing is probably low in this condition.

Macular hole during retinal detachment phase
Since there is retinal degeneration in these patients, the macula is likely to be thinner than normal in some cases. This may put trial participants at an increased risk of macular hole. In cases where a hole forms during the initial detachment phase, the hole should be closed by draining to air and closing the eye without administering vector. The patient can be brought back for vector administration at a later date, once the retina has healed. Gas in the eye lasts considerably longer in choroideremia patients and air or low concentration of sulphur hexafluoride gas (10%) should be used (Zingernagel et al., 2013).

Macular hole during vector injection phase
In this scenario, it is likely that vector will have escaped into the vitreous cavity, but since air will be used to close the hole, most will be taken back out of the eye which will reduce the risk of inflammation. Where possible, the surgeon should get as much vector suspension into the subretinal space as possible and then wait 20-30 minutes for absorption of vector from the subretinal space into the retinal pigment epithelium, before draining to air over the optic disc. The patient should be recovered if possible.
in the supine position and kept supine for 6-8 hours after surgery to allow maximal vector absorption from the subretinal space. Following that, the face down position can be adopted for a further 24 hours to flatten the retina and close the hole. Avoiding immediate face-down positioning gives more time for the vector suspension to be absorbed and the supine position limits movement of the eye which might otherwise vortex vector suspension out from the subretinal space into the vitreous cavity.

(ii) Immune reactions to vector capsid: These are not expected given the safety history with much larger doses of this vector in other clinical trials, but any immune reactions will be detected during the follow-up clinical examinations. Severe immune reactions triggered by the vector are likely to be evident within the first day of administration and might include severe vitritis, choroiditis or endophthalmitis. There may be additional systemic reactions such as headache and fever. Hence the patient will be monitored overnight and undergo a check up the day after surgery.

(iii) Immune reactions and/or toxic effects on retinal function caused by REP1: Due to the time required for transgene expression following subretinal delivery of AAV2, it is unlikely that any toxic effects of transgene product (REP1) will be evident until at least two weeks after surgery at the earliest. These might include similar immune reactions as described in (ii) above. Also any toxic effects of REP1 on visual function might be manifest by a worsening of visual acuity after the initial period of post-operative recovery. In practical terms any toxic effects on retinal function are likely to be manifest as a significant drop in visual acuity (defined by three or more ETDRS lines). Hence there are two post-operative visits (1-2 weeks and 1 month) when the patient will undergo ocular examination to detect for any inflammatory reactions.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse reaction, the nature or severity of which is not consistent with the applicable product information, which is documented in the Investigator’s Brochure. A SUSAR can be reported to the PEI (and other competent authorities, if applicable) and relevant ethics committees by either of the following methods:

1. Send a completed SUSAR Form with a cover letter (no standard format).
2. Send the completed SAE form (according to STZ eyetrial’s SOP) with a cover letter (no standard format). Ensure all the basic information required is included.

Reporting Procedures for all Adverse Events
All AEs occurring during the study observed by the investigator or reported by the participant, whether or not attributed to study medication, will be recorded on the CRF. The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to study medication, study procedure other
suspect drug or device and action taken. Follow-up information should be provided as necessary.

AEs considered related to the study medication/study procedure as judged by a medically qualified investigator or the sponsor will be followed until resolution or the event is considered stable. All related AEs that result in a participant’s withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs.

It will be left to the investigator’s clinical judgment whether or not an AE is of sufficient severity to require the participant’s removal from treatment. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of study assessment and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable. The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

- **Mild**: The subject is aware of the event or symptom, but the event or symptom is easily tolerated causing minimal discomfort to the subject
- **Moderate**: The subject experiences sufficient discomfort to interfere with or reduce normal everyday activities, but responds to symptomatic therapy or rest
- **Severe**: Significant impairment of functioning: the subject is unable to carry out normal everyday activities despite symptomatic therapy and/or the subject’s life is at risk from the adverse event

The relationship of AEs to the study medication/study procedure will be assessed by a medically qualified investigator and discussed with the PI. The causal relatedness may be defined as follows:

- **Unrelated**: is not reasonably related in time to the administration of the Investigational Medicinal Product (IMP) or exposure of the IMP has not occurred.
- **Unlikely to be related**: there are factors (evidence) explaining the occurrence of the event (e.g. progression of the underlying disease or concomitant medication are more likely to be associated with the event) or a convincing alternative explanation for the event.
- **Possibly related**: is clinically/biologically reasonably related to the administration of the IMP, but the event could have been due to another equally likely cause.
- **Probably related**: is clinically/biologically reasonably related to the administration of the IMP, and the event is more likely explained by exposure to the IMP than by other factors and causes.
- **Definitely related**: a causal relationship of the onset of the event, relative to administration of the IMP and there is no other cause to explain the event.

Any pregnancy occurring during the clinical study and the outcome of the pregnancy fathered by trial participants, should be recorded and followed up for congenital abnormality or birth defect.
Reporting Procedures for Serious Adverse Events

The Data Monitoring Committee (DMC) will undertake to review all SAEs for the study. The DMC is detailed in Appendix B and may hold electronic meetings. The DMC will meet at intervals and consider:

- Occurrence and nature of adverse events
- Whether additional information on adverse events is required
- Consider taking appropriate action where necessary to halt trials (see below)
- Act / advise on incidents occurring between meetings that require rapid assessment (e.g. SUSARs)

All SAEs will be reported to the DMC within one day of discovery or notification of the event. All SAE information will be recorded on an SAE form, which will be sent electronically to members of the DMC. Additional information received for a case (follow-up or corrections to the original case) will be detailed on a new SAE form. Once the SAE form is completed and signed by the PI, copies will also be sent to the Paul-Ehrlich-Institute. Fatal or life-threatening SUSARs will be reported within 7 days and all other SUSARs within 15 days. The PI will also inform all members of the trial team concerned of relevant information about SUSARs that could adversely affect the safety of participants.

In addition to the expedited reporting above, the PI shall submit an annual safety report to the Paul-Ehrlich-Institute and the EC.

7.3. Risk-Benefit Considerations

The risks of the subretinal vector injection are related to 1) the surgical procedure three port pars plana vitrectomy and subretinal surgery as well as 2) the ophthalmic or systemic effects of the rAAV2.REP1 vector.

Regarding 1) there may be a potential loss of visual function due to complications of the surgical procedure such as bacterial infection, retinal detachment, suprachoroidal and/or subretinal haemorrhage and cataract as a consequence of the surgical trauma. These risks are treatable by standard ophthalmological care but may result in lack of partial or complete restoration of the visual function loss.

Table 2 gives an overview of known risks by pars plana vitrectomy based on the literature and experiences from NHP trials with the vector of this study and estimates the rates to be expected in the current trial.

<table>
<thead>
<tr>
<th>Surgical complications during 23g PPV</th>
<th>Overall rate with mixed indications</th>
<th>Incidence in NHP study</th>
<th>Predicted risk in gene therapy trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>retinal detachment / breaks</td>
<td>1-10 %</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>endophthalmitis</td>
<td>0.01-1 %</td>
<td>0%</td>
<td>0.01-1 %</td>
</tr>
</tbody>
</table>
Table 2: Risks of complications during 23g PPV referring to literature, previous NHP studies and expected rates for the planned trial. [1] (Wilkinson et al., 2013) [2] (Lee et al., 2012a; Lee et al., 2012b) [3] (Wykoff et al., 2010).

<table>
<thead>
<tr>
<th>Complication</th>
<th>Expected Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound leakage / transient hypotony</td>
<td>5-20 %</td>
</tr>
<tr>
<td>Choroidal hemorrhage / detachment</td>
<td>&lt; 1 %</td>
</tr>
<tr>
<td>Suprachoroidal perfusion</td>
<td>&lt; 1 %</td>
</tr>
<tr>
<td>Vitreal haemorrhage</td>
<td>1-5%</td>
</tr>
<tr>
<td>Cataract formation</td>
<td>1-5%</td>
</tr>
<tr>
<td>Transient intraocular hypertension</td>
<td>5%</td>
</tr>
<tr>
<td>Corneal erosion</td>
<td>5-20%</td>
</tr>
</tbody>
</table>

Regarding 1) Cataract formation occurs more frequently and at an earlier time point in patients with retinal degeneration compared to healthy subjects. We therefore predict that the incidence of secondary cataract formation after pars plana vitrectomy is further increased in choroideremia patients. Cataract formation can be readily treated by surgery and recent evidence suggests that cataract surgery in choroideremia is indeed beneficial and safe (Edwards et al., 2015).

Regarding 2) there is a risk for a loss of vision due to ophthalmic immune reactions to the vector (indirect) or due to direct effects of the vector. However, no such reaction became apparent in the phase 1/2 trial (Maclaren et al., 2014). If any immune reaction should be triggered by the vector, this might include non-infectious inflammation of the pigment epithelium (epitheliitis), retina (retinitis), vitreous cavity (vitritis) or uveal tissue (uveitis). None of these have been reported in any previous clinical trial involving AAV in ocular gene transfer.

Such effects may result in a significant deterioration of visual function (decrease in VA of ≥ 15 letters) and might also occur after initial improvement. A vulnerable period for direct and/or indirect (immunogenic) effects due to the viral packaging protein of the vector would be the first two weeks (time of concomitant steroid treatment). No clinical trial has as of yet reported immune-reaction to the transgene (even in systemic gene transfer). It therefore seems unlikely that a direct or indirect effect due to transgene expression would occur after ocular gene transfer in the immune-privileged subretinal space.
Previous clinical trials with the same viral vector build have demonstrated good safety profiles (Nathwani et al., 2011; Maclaren et al., 2014). Other trials have shown good safety profiles after subretinal application of similar viral vector constructs (Bainbridge et al., 2008; Hauswirth et al., 2008; Maguire et al., 2008; Cideciyan et al., 2009; Maguire et al., 2009; Bennett et al., 2012; Jacobson et al., 2012). However, systemic risks cannot be completely ruled out and could stem from - previously undetected - immune reactions. Both the virus capsid and transgenic protein could potentially activate the immune system. The immune-privilege of the eye severely limits the likelihood of antigen presentation to leukocytes. The compartmentalization of the eye limits the biodistribution of viral vector. Much lower doses are required in the eye compared to previous studies targeting the liver (Nathwani et al., 2011). The transgenic protein is not secreted but expressed intracellularly. In light of these arguments and supported by results from previous clinical trials, it seems unlikely that a significant immune reaction is staged after intervention. Nevertheless, as immune reactions cannot be ruled out completely, patients will be screened accordingly during the follow-up clinical examinations. Any systemic reactions may appear during the first days as headache and fever. For safety reasons, patients will be hospitalized for up to three days including monitoring over the first night.

The potential of malignancy due to the AAV vector is also very unlikely. In contrast to e.g. lentiviral vectors, rAAV are considered non-integrating and result in episome formation in the transduced cells rather than integrating the transgene cassette in the host genome. Wild-type AAVs carry the complete virus genome including re Rep genes, which orchestrate the integration of vector DNA (preferentially at the AAVS1 locus (c19q13.3)). Deletion of AAV Rep in the recombinant vector reduces these integration events by more than 99.5% (Schnepp et al., 2003). So far, there has been no report on insertional mutagenesis of recombinant AAV (Lipinski et al., 2013). Nevertheless, all patients in this trial will be screened regularly even after study close out.
Dissemination of rAAV2.REP1 would most likely only occur between human beings, since it is derived from AAV2. However no replication is expected in normal cells of treated individuals exposed to the replication-deficient virus, or from exposure of uninfected people to treated individuals.

Germline transmission is theoretically possible but requires biodistribution far beyond what could be detected today combined with the extremely unlikely event of genomic integration.

Currently, there is no experience with the specific teratogenity of the rAAV2.REP1 vector, therefore the risk for an unborn child in case of an unforeseen conception, especially during the first half of the study participation, cannot be judged.

Generally, there is the potential of no subjective or objective benefit despite the risks of the procedure.
Patient benefits may consist in a deceleration or even halt of the disease and in the initiation of improved retinal function. For the individual patient this might become noticeable in improvement of night vision, retinal sensitivity, improved visual acuity and color vision. Improvements in visual functions are known to increase the vision-related quality of life and general well-being.

Risks of Steroid treatment:
Eye: increased intraocular pressure, glaucoma, cataract
Skin: thin skin, steroid acne, haemorrhage, prolonged wound healing, mouth dermatitis. Rarely hypersensitivity reactions, with for example rash.
Muscle and skeleton system: amyotrophia, osteoporosis, non-infectious bone necrosis
Psych: Depression, euphoria, increased appetite and impulsion, manifestation of latent epilepsy, increased intracranial pressure
Intestinal tract: Ulcers, haemorrhage, pancreatitis
Endocrinum: Adipositas, disturbed glucose metabolism, diabetes, edema, disturbed sexual hormone excretion
Blood circuit and vessels: Hypertension, increased risk for atherosklerosis thrombosis.
Blood, Immunesystem: increased leucocytes, decrease of lymphocytes, decrease of eosinophiles, general increase of blood cells, immunsuppression

7.4. Risks for the Environment

Dissemination of rAAV2.REP1 would most likely only occur between human beings, since it is derived from AAV2. However no replication is expected in normal cells of treated individuals exposed to the replication-deficient virus, or from exposure of uninfected people to treated individuals.

Specific safety countermeasures are not deemed necessary on the background of preclinical studies.

8 Examinations

8.1 Examinations before the patient enters the study

Screening and Eligibility Assessment
Patients with a clinical diagnosis of choroideremia and a molecular diagnosis of a null mutation in the gene encoding REP1 will be invited to take part in the study. In addition to the baseline visual tests outlined below, those eligible and giving informed written consent will also have their demographic information, medical history and concomitant medications recorded. They will also undergo a general examination to chart height, weight and temperature and measure resting pulse and blood pressure. Additional counselling about the implications of participating in the study will be provided by a suitably qualified non-medically qualified member of the research team.

8.2 **Examinations during the trial**

**Efficacy Measures**

There are several basic assessments of vision performed at various time-points during the study. First the clinical examination, which will include best corrected visual acuity and contrast sensitivity testing in addition to fundoscopy to check for signs of inflammation, cataract or retinal detachment. Additionally there are three anatomical assessments (fundus photography, autofluorescence and OCT scan) and functional tests (Goldman visual field, microperimetry, and colour vision).

**8.2.1 Best Corrected Visual Acuity**

Changes from baseline in visual acuity compared to the control eye is the study primary endpoint.

Patients will have a refraction test and assessment of visual acuity using the Early Treatment of Diabetic Retinopathy Study (ETDRS) vision charts in both eyes. This will be conducted in both eyes at the screening visit (Visit 1), pre-surgery on Day 0 (Visit 2), and at Visits 3-11 (and at ET). This examination will be performed at an unscheduled visit also.

**8.2.2 Fundus Autofluorescence**

The Spectralis HRT+OCT system will be used to record FAF, a measure of retinal pigment epithelial metabolism and viability (Schmitz-Valckenberg et al., 2008). Central 55° recordings will be made with optional extensions towards the outer limits of the treated area, if they are not covered by the central recordings. The untreated eye will be recorded first, followed by the treated one.

A change from baseline in autofluorescence evaluation is a secondary efficacy endpoint. Images will be captured for both eyes at screening (Visit 1) and at Visits 3-11 (and at ET). This examination will be performed at an unscheduled visit also.

**8.2.3 Microperimetry**

Microperimetry tests retinal sensitivity by projecting defined light stimuli to specific locations within the macula under mesopic conditions. The subjects are asked to press a button when a stimulus is perceived. Involuntary eye movements are
corrected in real-time and fixation stability is quantified. This test will be performed according to the Procedure Manual. Changes from baseline in microperimetry readings is a secondary efficacy endpoint. Microperimetry will be conducted at least three times pre-operatively to reduce learning effects and with the fellow eye as a control. Microperimetry will be conducted at screening (Visit 1) and from Visit 4-11 (and at ET). This examination will be performed at an unscheduled visit also.

8.2.4 Spectral Domain Optical Coherence Tomography (SD-OCT)

Patients will be examined in mydriasis using a Spectralis HRT+OCT system (Heidelberg Engineering, Heidelberg, Germany). The patient is asked to look at a visual cue while eye-tracking software will correct for involuntary eye movement. SD-OCT will be performed in both study and non-study eye at the screening visit (Visit 1) and Visit 3-11 (and at ET). This is a secondary efficacy measure that will quantify reduction in the signal from the outer nuclear layer and choroid in the treated eye compared to the fellow control eye. Foveal changes will also be assessed carefully because progressive foveal thickening has been noted in the early phase of the disease (Jacobson et al., 2006). This examination will be performed at an unscheduled visit also.

8.2.5 Visual Fields

Conducted in both eyes at screening (Visit 1) and Visits 9 and 11 (and at ET) utilizing Goldmann perimetry. Visual fields will be assessed at least three times pre-operatively to reduce learning effects and with the fellow eye as a control. Change from baseline will be evaluated for each eye in all 6 patients by an appropriately qualified masked observer.

8.2.6 Contrast Sensitivity

This test for contrast sensitivity will be performed in both eyes at standardized illumination conditions, according to the Procedure Manual of the Pelli Robson (PR) chart. In contrast to the user instruction of the PR chart, the test distance will be 3m (instead of 1m), as own data have shown, that the standard distance of 1m is inferior in sensitivity. This test will be conducted at screening (Visit 1), Visit 5-11 (and at ET).

8.2.7 Colour Vision

The Roth 28 test will be used at screening (Visit 1) and Visits 5-11 (and at ET) to assess colour vision in both eyes. In addition, other colour vision tests may be used if deemed appropriate for each patient (e.g Cambridge Colour Vision test, FM-100).

Safety Measures
8.2.8 Full Ophthalmic Examination

The full ophthalmic exam will be used as a safety assessment throughout the study and will include intraocular pressure, slit lamp examination, lens opacity grading and dilated ophthalmoscopy examination. This examination will be conducted in both eyes at the screening visit (Visit 1), pre-surgery on Day 0 (Visit 2) and at Visits 3-11 (and at ET). This examination will be performed at an unscheduled visit also. The same slit lamp machine and lighting conditions should be used across study visits for a given patient.

In addition to the parameters listed above, patients should be examined carefully for the presence of intraocular inflammation after vector administration. Cataract can also develop as a result of the vitrectomy procedure and could potentially affect visual acuity. Pre-operative grading of lens opacity and colour should therefore be documented by the established clinical lens opacities classification system (LOCS). A recent study has shown that cataract surgery is effective in patients with choroideremia and without any specific risks (Edwards et al., 2015). Hence cataract surgery will be offered at the appropriate time if the cataract is deemed to be visually significant.

8.2.9 7 field colour fundus photography

Conducted in both eyes at screening (Visit 1) and Visits 7, 9-11 (and at ET).

Standard sequential colour fundal photographs will be used to aid objective clinical assessment of progressive retinal changes and will be compared between both eyes at baseline and at different timepoints.

8.2.10 Fluorescein and Indocyanine Angiography

Fluorescein and indocyanine angiography will be conducted in both eyes at screening (Visit 1) and Visit 11 (and at ET).

ICG Angiography

Indocyanine Green is a dye, which fluoresces in the infra-red light. The infra-red waves have the ability to penetrate the retinal layers making the circulation in deeper layers visible when photographed with an infra-red sensitive camera. The dye is applied by intravenous injection and flows through the body to reach the choroidal and retinal circulation. Due to its nature stays in the retinal and choroidal vessels, this allows to see and identify the distinct outlines of the vessels of the choroid.

Fluorescein Angiography (FA)

Perfusion characteristics will be assessed in mydriasis using a wide-angle objective after intravenous bolus administration of 10% fluorescein sodium (500mg in 5ml; Novartis Pharma AG, Bern, Switzerland).

The central fundus of the treated eye will be recorded with sequential photographs in the first 45 seconds after injection. At 1 and 5 minutes after injection the same 9-view recordings will be performed in both eyes.
8.2.11 Vital signs

Resting pulse blood pressure and body temperature will be taken at screening (Visit 1) and Visit 3 - 11 (and at ET), as part of routine safety monitoring of the patient throughout the study.

8.2.12 Blood chemistry

Blood samples will be taken for urea, electrolytes and liver function tests at screening (Visit 1), Visit 6, 7, 9 and 11 (and at ET) as part of routine safety monitoring.

8.2.13 Viral Shedding

Blood, tears, saliva and urine samples will be collected and tested by PCR amplification of vector genomes to assay for evidence of vector shedding and dispersion. Samples will be taken at screening (Visit 1), and Visits 3-6.

8.2.14 Immunogenicity

8.2.14.1 Immunoassay

Blood will be taken to assess antibody and cell based responses against rAAV2-REP1. Samples will be taken at screening (V1) to assess baseline T-cell mediated responses and baseline levels of pre-existing antibodies to AAV2. Post injection blood samples will be taken at Visit 4-7, Visit 9 and Visit 11 (and at ET) to further assess T-cell mediated responses as well as antibody responses to the AAV2 capsid and REP1 protein. ELISPOT assays will be used for T-cell mediated immune responses and antibody responses will be assayed using ELISA based methods. Methods are described in more detail in the following:

Antibody Assays
The assays described below are currently in development and are based on published literature methods. The assays may be subject to change due to the specific nature of the analytes and availability of reagents and standards.

Anti-AAV2 Antibody ELISA
Due to the expectation that patient samples will contain pre-existing anti-AAV2 antibodies samples will be serially diluted and an end point titre value reported. Microtitre plates will be coated with AAV2. Plates will be blocked to reduce non-specific binding before addition of serial dilutions of the patient serum samples. Following incubation to allow anti-AAV2 antibodies to bind, a detection reagent will be added. The nature of the detection reagent will be dependent on the positive and negative assay controls used; if human positive and negative control serum samples can be identified by screening of healthy individual donors an anti-human IgM/IgG conjugated (e.g. HRP) antibody will be used. If animal antibody controls are used a non-species specific detection reagent (e.g. protein A/G-HRP) will be used. An
appropriate substrate (e.g. TMB) for the detection reagent will be added, the reaction stopped and the signal measured using a plate reader. The end point titre will be defined as the dilution at which the signal is diluted to $\leq$ background.

**Anti-AAV2 Neutralising Antibody Assay**

Samples found to be positive in the anti-AAV2 ELISA will be analysed in cell-based neutralising antibody assay. In the proposed assay format, serum samples will be incubated with AAV2 containing a marker (e.g. luciferase, Green Fluorescent Protein). The samples will be added to a cell line (e.g. HEK 293) and following washing the degree of uptake of the marker in the cells will be measured using a plate reader or by flow cytometry. Inhibition of uptake will be indicative of the presence of anti-AAV neutralising antibodies. Positive/negative samples will be assessed against a cut-point to take non-specific binding into account.

**Anti-REP1 Antibody ELISA**

The anti-REP1 assay will be developed using a tiered approach typically used for the assessment of unwanted immunogenicity for therapeutic proteins i.e. screening, confirmatory and neutralising activity. A bridging ELISA format is proposed where the antigen, REP1, is used or capture and a labelled (e.g. biotin) form of the antigen is used for detection. This assay format removes the requirement for a species specific positive control. Briefly, microtitre plates will be coated recombinant human REP1. Following blocking to reduce non-specific binding patient samples will be added. Any anti-REP1 antibodies present in the samples will bind to the REP1 coated to the plate. Following washing to remove non-specific binding the plates bound antibodies will be detected by the addition of the labelled REP1 (e.g. REP1-biotin) following by the appropriate substrate (e.g. streptavidin-HRP). The colour development reaction stopped and the signal measured using a plate reader.

In the screening assay samples will be analysed at a single dilution and the measured values assessed against an assay cut-point statistically determined from the analysis of control sera (mean ± 1.645 standard deviations). Any samples greater than or equal to the cut-point will be considered positive. This cut-point should yield at least 5% false positive results along with any true positive results. Any screening positive samples will be then analysed in a confirmatory assay; this will use the same ELISA format but the samples will be pre-incubated with REP1. Samples whose signals are decreased by the pre-incubation will be considered as true positive results.

If confirmed positive samples are found they will be subject to analysis in a cell-based neutralising antibody assay.

**Cellular Immune Response Assay – ELISPOT**

Cellular immune responses against AAV2 and REP1 will be assessed using an Enzyme Linked Immuno Spot (ELISPOT) assay. Peripheral blood mononuclear cells (PBMCs) will be isolated from whole blood at site and stored frozen in liquid nitrogen prior to analysis. Briefly, PBMC samples mixed with the potential antigens, in this case AAV2 and REP1 peptides, prior to addition to microtitre plates containing a membrane coated with capture antibody against a T-cell secretory cytokine (e.g interferon-γ). Cells activated by the antigens secrete the cytokine which is captured
by the antibody on the membrane. Following washing, captured cytokines are visualised by the addition of secondary labelled detection antibodies and the substrate. Captured cytokines are visualised as coloured spots which are then counted using an ELISPOT plate reader. In the absence of true positive controls, common antigens which virtually all of the population have been exposed to (e.g. influenza, CMV and EBV) will be analysed with all samples along with Concanavalin A, a potent stimulator of T-cells, to ensure the assay is performing acceptably.

8.2.14.2 Immunochemistry

Blood will be taken to assess CRP levels, as well as total IgM and total IgG at screening (Visit 1) as well as Visits 4-7, Visit 9 and Visit 11 (and at ET). The immunology tests represent part of the general safety monitoring of the study and may also provide important background data for future retreatment or application of vector to the fellow eye, but are not specified as trial endpoints. Additional tests on vector shedding will be performed on saliva, urine and tears throughout the study. The 1 month test is to establish a post-operative baseline in case there are significant changes in the retina caused by the surgical procedure. The 6 month check is to identify any obvious early detrimental effects to expression of REP1 by the AAV vector. The 12 month assessments would also be used to establish any future trend, as well as to exclude any medium term detrimental effects of the vector. The 24 month checks would define the end point of the study and the data here would be used to assess efficacy of the vector in terms of primary and secondary endpoints.

8.3 Follow up

Annual checks would continue thereafter with additional tests performed at the discretion of the PI. In particular these checks would include screening for malignancy and any emerging safety concerns would be reported directly to the authorities.

8.4 Detailed schedule of visits and examinations

Visit 1 Screening

- Informed consent
- Demography, medical and ocular
- Vital Signs (Pulse, Blood pressure, Body temperature)
- Blood chemistry
- Viral shedding
- Immunochemistry
- Immunoassay
- BCVA
• Full ophthalmic exam
• SD-OCT
• Autofluorescence
• Microperimetry
• Contrast sensitivity
• Colour vision
• Fluorescein angiography
• Indocyanine angiography
• 7-field colour fundus photos
• Visual fields
• AE/SAE monitoring
• Concomitant medication

Visit 2
Day 0, Injection Day
• Full ophthalmic Exam
• Study drug/subretinal injection
• AE/SAE monitoring
• Concomitant medication

Visit 3
Day 1 post op
• Vital signs (pulse, blood pressure, body temperature)
• Viral shedding
• Full ophthalmic exam
• SD-OCT
• Autofluorescence
• AE/SAE Monitoring
• Concomitant Medication

Visit 4
Day 7 ± 3d
• Vital signs (pulse, blood pressure, body temperature)
• Basic ophthalmic exam
• Scanning laser ophthalmoscopy
• Optical coherence tomography
• Haematology, basic chemistry and immunology
• Oral steroids
• Antibiotic & anti-inflammatory topical medication
• AE/SAE monitoring
• Concomitant medication

Visit 5
Month 1 ± 3d
- Vital signs (pulse, blood pressure, body temperature)
- Viral shedding
- Immunochemistry
- Immunoassay
- BCVA
- Full ophthalmic exam
- SD-OCT
- Autofluorescence
- Microperimetry
- Contrast sensitivity
- Colour vision
- AE/SAE monitoring
- Concomitant medication

Visit 6
Month 3 ± 7d
- Vital signs (pulse, blood pressure, body temperature)
- Blood chemistry
- Viral shedding
- Immunochemistry
- Immunoassay
- BCVA
- Full ophthalmic exam
- SD-OCT
- Autofluorescence
- Microperimetry
- Contrast sensitivity
- Colour vision
- AE/SAE monitoring
- Concomitant medication

Visit 7
Month 6 ± 7d
- Vital signs (pulse, blood pressure, body temperature)
- Blood chemistry
- Viral shedding
- Immunochemistry
- Immunoassay
- BCVA
- Full ophthalmic exam
- SD-OCT
- Autofluorescence
- Microperimetry
- Contrast sensitivity
- Colour vision
- 7-field colour fundus photos
- AE/SAE monitoring
- Concomitant medication

**Visit 8**  
**Month 9 ± 14d**
- Vital signs (pulse, blood pressure, body temperature)
- BCVA
- Full ophthalmic exam
- SD-OCT
- Autofluorescence
- Microperimetry
- Contrast sensitivity
- Colour vision
- AE/SAE monitoring
- Concomitant medication

**Visit 9**  
**Month 12 ± 14d**
- Vital signs (pulse, blood pressure, body temperature)
- Blood chemistry
- Viral shedding
- Immunochemistry
- Immunoassay
- BCVA
- Full ophthalmic exam
- SD-OCT
- Autofluorescence
- Microperimetry
- Contrast sensitivity
- Colour vision
- 7-field colour fundus photos
- Visual fields
- AE/SAE Monitoring
- Concomitant medication

**Visit 10**  
**Month 18 ± 28d**
- Vital signs (pulse, blood pressure, body temperature)
- BCVA
- Full ophthalmic exam
- SD-OCT
- Autofluorescence
- Microperimetry
- Contrast sensitivity
- Colour vision
- 7-field colour fundus photos
- AE/SAE Monitoring
- Concomitant Medication

**Visit 11 Close-out**  
**Month 24 ± 28d**

- Vital signs (Pulse, blood pressure, body temperature)
- Blood chemistry
- Viral shedding
- Immunochemistry
- Immunoassay
- BCVA
- Full ophthalmic exam
- SD-OCT
- Autofluorescence
- Microperimetry
- Contrast sensitivity
- Colour vision
- Fluorescein angiography
- Indocyanine angiography
- 7-field colour fundus photos
- Visual fields
- AE/SAE monitoring
- Concomitant medication

**Early Termination (ET)**

- Vital signs (pulse, blood pressure, body temperature)
- Blood chemistry
- Viral shedding
- Immunochemistry
- Immunoassay
- BCVA
- Full ophthalmic exam
- SD-OCT
- Autofluorescence
- Microperimetry
- Contrast sensitivity
- Colour vision
- Fluorescein angiography
- Indocyanine angiography
• 7-field colour fundus photos
• Visual fields
• AE/SAE monitoring
• Concomitant medication

**Unscheduled Visit**

• BCVA
• Full ophthalmic exam
• SD-OCT
• Autofluorescence
• Microperimetry
• AE/SAE monitoring
• Concomitant medication

### 9 Documentation of results in source documents

The responsible investigator or member of the trial team will document all relevant information from every patient who enters the study in the provided worksheets and validate it with his stamp and signature. From the electronic patient file (AIS) only the informed consent procedure will be printed out, signed and dated. All information must be complete and plausible. All entries must be clear and legible. Every alteration must be carried out according to the following rules:
- the old version has to be crossed out so that it remains still readable
- the new version has to be written above or beside the old one
- the correction needs to be verified by adding the date, initials and possibly the reason

### 10 Biometrical planning and analysis

#### 10.1 Trial design

Open label trial.

#### 10.2 Sample size calculation

**The Number of Participants**

Six patients will be enrolled into this study. No formal sample size calculations have been performed.
10.3 Definition for study groups

Study populations are defined in the following subsections. All populations will be identified and finalized in the Statistical Analysis Plan.

Safety Population

The Safety Population is defined as all subjects who receive at least one dose of investigational product or corticosteroids and have at least one post-therapy safety assessment.

Intent to Treat Population

The Intent to Treat (ITT) population is defined as all subjects who are randomised and received at least one dose of investigational product or corticosteroids, and for whom at least one post-baseline assessment is available.

Per Protocol Population

The Per Protocol (PP) Population is a subset of the ITT Population and consists of subjects who do not have any major protocol violations. The criteria for defining the PP set will be fully defined in the Statistical Analysis Plan.

A final decision for the allocation to the different populations of the subjects will be made before analysis of trial results.

10.4 Analysis methods

Statistical and Analytical Plans

Summary statistics will be presented for both eyes (treated eye versus control eye groups). No formal statistical comparison will be performed (no p-value will be computed). For categorical/binary data, the number and proportion of patients pertaining to each category will be presented with its 95% Confidence Interval (CI). For continuous data, mean (and its 95% CI) and Standard Deviation (SD) will be presented.

The primary outcome measure will be the proportion of patients with a relative change from baseline of > 5 in ETDRS letters, when comparing a patient’s treated eye versus the control eye. At each time point, the change from baseline in ETDRS letters will be computed for each eye. The mean change from baseline in ETDRS letters will be presented for, both, the Treated Eye and the Control Eye groups.
At each time point, the change from baseline and the percentage change from baseline in the area of autofluorescence will be computed for each eye and their mean will be presented for, both, the Treated Eye and the Control Eye groups.

With regards to microperimetry, at each time point, the change from baseline in mean sensitivity will be computed for each eye. The mean change from baseline in mean sensitivity will be presented for, both, the Treated Eye and the Control Eye groups.

Adverse events will be listed.

Other Investigator Sponsored Studies are expected to be run with a similar protocol. A meta-analysis on the Investigator Sponsored studies is planned. A separate Statistical Analysis Plan describing the details of the meta-analysis will be developed.

10.5 Interim analysis and break-off criteria

Stopping rules: A single occurrence of any one of these events would halt the study

- Severe ocular inflammation that is unresponsive to treatment (endophthalmitis).
- Any suspected unexpected serious adverse reaction (SUSAR).
- At the request of the Data Monitoring Committee, based on safety concerns.

If stopping rules are applied, the DMC should convene an extraordinary meeting to decide if the study should continue. In most cases this will involve assessment of probability that the stopping rule was triggered by a serious adverse reaction that is likely to be repeated in subsequent trial participants.

11 Conditions for Amendments

In case of necessary changes to the flowchart of visits and procedures or other aspects of the trial, an amendment will be submitted to PEI and EC. Those changes will not be implemented unless approved by the PEI and EC. An exemption is changes to the protocol preventing immediate hazard for the patient. In this case implementation may happen before approval. Any amendment will result in a revised version of the patient information, which needs to be signed by all patients. Also a recommendation of the DMC may result in an amendment to the trial protocol.

12 Ethical and Regulatory Aspects

The UKT as Sponsor will be responsible for the overall conduct of the clinical trial and will be responsible for ensuring the trial is conducted according to the protocol and all regulatory requirements and regulations.

The protocol and informed consent form for this study must be approved by an appropriately constituted EC as defined by local requirements. The list of the EC voting members, their titles or occupation, and their institutional affiliations and/or the EC general assurance number, if applicable, must be provided with the approval. The EC will also be notified of completion of the study and a final report must be submitted to the EC and the PEI in accordance with the requirements.
Investigator will maintain an accurate and complete record of all communication, reports and submissions to the PEI and EC.

This trial underlies the German AMG in its current version and will be submitted, performed and monitored according to ICH-GCP standards. Any study procedure will only be performed after the patient has given written informed consent. The informed consent will be documented in the electronic patient file of the Centre for Ophthalmology (AIS) giving the date of consent, study name and the name of the physician. Each subject's signed informed consent must be kept on file by the Investigator.

German data protection regulations will be followed and patients will be informed in a standardized and detailed manner about pseudonymized health data and those persons/institutions who will have access to personal data under special restrictions according to law.

The Sponsor of this trial is UKT (Universitätsklinikum Tübingen) represented by the Principal Investigator. Defined tasks have been delegated to the CRO of the trial, STZ eyetrial at the Centre for Ophthalmology, University of Tübingen such as, but not limited to, communication with regulatory authorities, submission of the trial to PEI and EC, monitoring of the trial and safety reports. The documents used for submission and conduct of this trial will be according to the SOP system of the local CRO, STZ eyetrial at the Centre for Ophthalmology, which is a certified member of the EVICR.net.

UKT has contracted insurance for trial participation as well as travel insurance. The contact dates of the patient insurance are:
Bernhard Hoppe
HDI-Gerling Industrie Versicherung AG
Niederlassung Düsseldorf
Vertragsservice Haftplicht
Am Schönenkamp 45
40599 Düsseldorf
Tel.: +49 (0)211 7482-5404
Fax: +49 (0)511 645-1150023

Contact dates for the travel insurance for participating patients are:
Axel Tunsch
Sachbearbeiter
Haftplicht Betrieb AD/Sparkassen und Unfall (FS51)
SV SparkassenVersicherung
Bahnhofstraße 69
65185 Wiesbaden
Telefon: 0611 178 -2531
Telefax: 0611 178 - 12531
The amount covered for the patient insurance is 500 000€ per patient. The amount covered for the travel insurance is 100 000€ for invalidity und 50 000€ in the case of death.

The patient has the obligation to immediately inform the insurance companies mentioned above, if necessary with the support of the Investigator. The patient also has to inform the investigator.

All patients will receive a copy of the insurance police, along with the copy of the patient informed consent.
13 References


APPENDIX A: DATA MONITORING COMMITTEE (DMC) CHARTER

13.1 Introduction
The trial, “THOR - Tübingen Choroideremia gene therapy trial open label Phase 2 clinical trial using an adeno-associated viral vector (AAV2) encoding Rab-escort protein 1 (REP1)” is funded by Tistou & Charlotte Kerstan Foundation. The trial is registered with the European Union Drug Regulating Authorities Clinical Trials; reference EudraCT 2014-005004-21.

13.2 Trial objectives
This is the first German ophthalmological gene therapy trial in Choroideremia that will assess the effects of an adeno-associated viral (AAV) vector expressing the REP1 transgene in the treatment of a retinal degeneration known as choroideremia. The REP1 gene (Xq21.2) is deficient in choroideremia, resulting in a slow degeneration leading to blindness in affected patients (in most cases male). Currently there is no effective treatment for this disease. The primary endpoint of the clinical trial is change from baseline in best corrected visual acuity in the treated eye, compared to the untreated control eye. Secondary endpoints are absence of vector related adverse reactions and demonstration of improved retinal anatomy and/or visual function other than best corrected visual acuity in the treated eye compared to the untreated control eye 24 months after vector administration.

13.3 Scope
The purpose of this document is to describe the roles and responsibilities of the independent DMC for CHOR-TUE-01, including frequency and format of meetings, methods of providing information to and from the DMC, statistical issues and relationships with other committees.

Roles and responsibilities

13.4 Aims
To protect and serve CHOR-TUE-01 participants, in particular with regard to safety and to assist and advise Principal Investigators so as to protect the validity and credibility of the trial. To safeguard the interests of trial participants, assess the safety and efficacy of the gene therapy intervention during the trial, and monitor the overall conduct of the clinical trial.

13.5 Terms of reference
The DMC should receive and review the progress and accruing data of the CHOR-TUE-01 trial and provide advice on the conduct of the trial to the Trial Steering
Committee (the Principal Investigators listed on Page 1). The DMC should inform the Principal Investigator if there is a consensus that the ongoing results show that the medicinal product (or its method of administration) are no longer in the best interests of trial participants.

13.6 Specific roles of DMC

Interim review of trial progress, including updated figures on data quality, main outcomes and safety data. This review would include, but not be restricted to, the following:

- assess data quality, including completeness (and by so doing encourage collection of high quality data)
- monitor recruitment figures and losses to follow-up
- monitor compliance with the protocol by participants and investigators
- monitor evidence for treatment differences in the main efficacy outcome measures
- monitor evidence for treatment harm (e.g. toxicity data, SAEs, deaths)
- decide whether to recommend that the trial continues to recruit participants or whether recruitment should be terminated either for everyone or for some treatment groups and/or some participant subgroups
- suggest additional data analyses
- monitor planned sample size assumptions
- monitor continuing appropriateness of patient information
- monitor compliance with previous DMC recommendations
- consider the ethical implications of any recommendations made by the DMC
- assess the impact and relevance of external evidence

13.7 DMC roles prior to starting the trial

All potential DMC members should have seen the protocol before agreeing to join the committee. Therefore, if a potential DMC member has major reservations about the trial (e.g. the protocol or the logistics) they should report these to the Principal Investigator and may decide not to accept the invitation to join. DMC members should be independent and constructively critical of the ongoing trial, but also supportive of aims and methods of the trial.
13.8 Timing of DMC meetings
The first meeting of the DMC should be scheduled prior to the first injection of vector, to discuss the protocol, the trial, any analysis plan, future meetings, and to have the opportunity to clarify any aspects with the Principal Investigators, and to discuss the format of reports to be submitted to the DMC. The meeting may be conducted electronically (by email) or telephone conference. Several subsequent meetings of the DMC will be held (see Figure 2 on page 23). A final meeting should be scheduled after all trial participants have been treated with vector, predicted to be within two years of the trial start date.

Composition

13.9 DMC Membership
The DMC members are independent of the trial (that is, they are not involved with CHOR-TUE-01 in any other way, or have a competing interest that could impact on the trial). Any competing interests, both real and potential, should be declared. A short competing interest declaration should be completed by each of the DMC members and returned to the Principal Investigator, Dominik Fischer at the University of Oxford. Three members have agreed to form the DMC for CHOR-TUE-01:

1) Eberhart Zrenner, Tübingen
2) Peter Issa Charbel, Bonn
3) Camiel JF Boon, Leiden

13.10 The responsibilities of the clinical trial Investigators to the DMC
The Principal Investigator (Dominik Fischer), may be asked, and should be available, to attend open sessions of the DMC meeting. Other CHOR-TUE-01 Principal Investigators will not usually be expected to attend, but can attend sessions when necessary. The Principal Investigator will provide a report for the DMC at least two weeks prior to the scheduled DMC meeting. In addition to providing interim data, the report will also detail any AEs or ARs. All SAEs will be reported to the DMC within one day of discovery or notification of the event. All SAE information will be recorded on an SAE form, which will be sent electronically to members of the DMC. Additional information received for a case (follow-up or corrections to the original case) will be detailed on a new SAE form. The PI will also report all SUSARs to the Regulatory Authorities and the Ethics Committee. Fatal or life-threatening SUSARs will be reported within 7 days and all other SUSARs within 15 days. The PI will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

13.11 Advisory role of DMC
As is customary, the CHOR-TUE-01DMC will not make decisions about the trial, but rather make recommendations from the DMC Chair to the Principal Investigator.
13.12 DMC payments

CHOR-TUE-01 DMC members will be reimbursed for travel and accommodation. No other payments or rewards are anticipated.

13.13 DMC members and competing interests

Competing interests should be disclosed. These are not restricted to financial matters – involvement in other trials or intellectual investment could be relevant. Although members may well be able to act objectively despite such connections, complete disclosure enhances credibility. DMC members should not use interim results to inform trading in pharmaceutical shares, and careful consideration should be given to trading in stock of companies with competing products.

13.14 DMC meetings format

After discussion of the report and any additional data (such as might come to light from other ongoing clinical trials), the DMC will decide on a recommendation, which could include:

- No action needed, trial continues as planned
- Early stopping rule, due for example, to clear harm of treatment
- Stopping recruitment within a subgroup
- Extending recruitment (where results are equivocal for six patients)
- Sanctioning and/or proposing protocol changes

The DMC will submit these recommendations in writing to the Principal Investigator within one week of conclusion of the meeting.

13.15 DMC decision making methods

It is recommended that every effort should be made for the DMC to reach a unanimous decision. If the DMC cannot achieve this, a vote may be taken, although the role of the Chair is to summarise discussions and encourage consensus; it may be best for the Chair to give his own opinion last. All three members need to take part in the DMC meeting in order to constitute a quorum.

Final DMC meeting

At the end of the trial, assuming there have been no extraordinary meetings (i.e. following a SUSAR), the fourth and final DMC meeting will allow the DMC to discuss all the trial data with the Principal Investigator and make recommendations for data interpretation.