# SAFETY REPORT ADVERSE EVENTS

## Protocol: THOR-TUE-01

## 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)

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|---|---|
| **Investigational Product:** | rAAV2.REP1 |
| **Protocol Number:** | THOR-TUE-01 |
| **Version Number:** | 2.0 |
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| **Phase:** | II |
| **Report Number:** | 1.0 |
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# SYNOPSIS

**Title:** 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:** 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:** 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 (Study Eye)**

- Participant is willing and able to give informed consent for participation in the study.
- Male aged 18 years or above.
- 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.
- Active disease visible clinically within the macula region
- 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**

- Female and child participants (under the age of 18)
- Participants with a history of amblyopia in the study eye
- Men unwilling to use barrier contraception methods, if relevant

- 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.
- 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.
- 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.
- Patients with amblyopic eyes should be excluded in general, since the evaluation of the primary endpoint presupposes the ability to fixate both eyes
- Prior intraocular surgery within six months
- Intolerance to local anesthesia and/or contraindication to IVT surgery (anemia Hb<8g/dl, severe cardiovascular disease, severe coagulopathy, etc.)
- 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)
- Patients suffering from other genetic mutations leading to pathological retinal conditions
- 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 1011 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 autofluoresence 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.

**Time Schedule:**
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.

# 1.0 PREVIOUS REPORTED SIDE EFFECTS

| Study Type | Vector | Model | AEs | SAEs | Author |
|---|---|---|---|---|---|
| Clinical trial | rAAV2 | Human (n=3); RPE65; LCA | - Mild self-limiting postoperative intraocular inflammation<br>- Small increase in non-specific activation of T-cells (n=2) | none | Bainbridge et al., 2008 |
| Clinical trial | rAAV2 | Human (n=3); RPE65; LCA | - Foveal thinning (n=1) | none | Hauswirth et al., 2008 |
| Clinical trial | rAAV2 | Human (n=3); RPE65; LCA | - Macular hole (n=1) | none | Maguire et al., 2008 |
| Clinical trial (phase 1) | rAAV2 | Human (n=15); RPE65; LCA | - Retinal detachment with surgical repair (n=1)<br>- Choroidal effusion (n=1)<br>- Ocular hypotension (n=4)<br>- Ocular hypertension due to topical steroids (n=3) | none | Jacobson et al., 2012 |
| Clinical trial, long-term follow-up | rAAV2 | Human (n=5); RPE65; LCA | - Macular hole (n=1) | none | Testa et al., 2013 |
| 2 clinical trials (phases 1/2 and 2) | rAAV2 | Human (n=18); CHM gene (REP1); Choroideremia | - n=96<br>- 36/96 drug- or procedure-related<br>- 5/96 related to investigational product | n=1 (Presence of air bubble during surgery) | Investigator´s Brochure "THOR Study" by NightstaRx Ltd; 09 Nov 2015 |

***Table 1:*** *Reported Adverse events and serious adverse events in previous clinical trials with the same vector or similar vector constructs. Abbreviations: AE = Adverse event; SAE = Serious adverse event; RPE = Retinal pigment epithelium;*
*rAAV2 = recombinant adeno-associated virus serotype 2; LCA = Leber´s congenital amaurosis.*

Summary:
During previous clinical trials in patients with either Lebers congenital amaurosis or Choroideremia only one serious adverse events (SAE) was reported.
Of the documented adverse events (AE) only five were classified as related to the investigational product (vector).

## 2.0 RISK-BENEFIT-CONSIDERATIONS PER PROTOCOL 2.0 FROM 09 November 2015

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.

| **Surgical complications during 23g PPV** | Overall rate with mixed indications [1-3] | Incidence in NHP study | Predicted risk in gene therapy trial |
|---|---|---|---|
| retinal detachment / breaks | 1-10 % | 0% | 1% |
| endophthalmitis | 0.01-1 % | 0% | 0.01-1 % |
| wound leakage / transient hypotony | 5-20 % | 100% | 5-20 % |
| choroidal hemorrhage / detachment | < 1 % | 0% | < 1 % |
| suprachoroidal perfusion | < 1 % | 0% | < 1 % |
| vitreal hemorrhage | 1-5% | 0% | < 1 % |
| cataract formation | 1-5% | 9% | 10-20% |
| transient intraocular hypertension | 5% | 0% | 5% |
| corneal erosion | 5-20 % | 5-20 % | 5-20 % |

***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., 2012 [3] Wykoff et al., 2010.*

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

Psyche: 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 circiut and vessels: Hypertension, increased risk for atherosklerosis thrombosis.
Blood, Immunesystem: increased leucocytes, decrease of lymphocytes, decrease of eosinophiles, general increase of blood cells, immunosuppression

**Risks for the Environment**
These are dealt with in detail in the separate ERA document *Information required concerning releases of genetically modified organisms* according to Annex IIIa of the DIRECTIVE 2001/18/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.

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.

# 3.0 REPORTED SERIOUS ADVERSE EVENTS (SAE) IN THOR-TUE-01

No serious adverse events (SAE) have been reported.

# 4.0 REPORTED ADVERSE EVENTS (AE) IN THOR-TUE-01

From beginning of the study in January 2016, 18 adverse events in 6 patients were reported. Ten adverse events were non-ocular, eight were ocular. Two adverse events are currently unresolved (marked with * in the list below).

For the eight ocular adverse events a plausible relationship to study procedure was documented. None of the adverse events was classified as related to study drug (vector).

**List of reported adverse events in THOR-TUE-01**

| | |
|---|---|
| Non-ocular adverse events | |
| Gastrointestinal infection | 2 |
| Common cold | 2 |
| Knee abrasions | 1 |
| Sore throat after inoculation | 1 |
| Fall | 1 |
| High blood glucose | 1 |
| Urinary tract infection | 1 |
| Headache | 1 |
| Ocular adverse events | |
| Double Vision | 1 |
| Worsening of diplopia | 1* |
| Blurred vision | 1 |
| Macular hole | 1* |
| Residual neurosensory detachment | 1 |
| Punctate bleeding at retinotomy | 1 |
| Conjunctival redness | 2 |

* *unresolved*

## 5.0 APPRAISAL OF THE RISK-TO-BENEFIT RATIO

No SAEs occurred so far during the study. For all of the ocular AEs it can be considered that they are related to the surgical procedure itself and not to the adenovirus-associated vector (AAV). There are no AEs outside the expected side effect profile of the study procedure and study drug. Thus, regarding safety no protocol changes are required. As a consequence of this report, the risk-to-benefit considerations as documented in the protocol remain unchanged.

Date: 11.08.2017

Principal Investigator FISCHER

| Name | Signature |
|---|---|