

# BMJ Open Treatment of optic neuritis with erythropoietin (TONE): a randomised, double-blind, placebo-controlled trial—study protocol

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## ABSTRACT

**Introduction:** Optic neuritis leads to degeneration of retinal ganglion cells whose axons form the optic nerve. The standard treatment is a methylprednisolone pulse therapy. This treatment slightly shortens the time of recovery but does not prevent neurodegeneration and persistent visual impairment. In a phase II trial performed in preparation of this study, we have shown that erythropoietin protects global retinal nerve fibre layer thickness (RNFLT-G) in acute optic neuritis; however, the preparatory trial was not powered to show effects on visual function.

**Methods and analysis:** Treatment of Optic Neuritis with Erythropoietin (TONE) is a national, randomised, double-blind, placebo-controlled, multicentre trial with two parallel arms. The primary objective is to determine the efficacy of erythropoietin compared to placebo given add-on to methylprednisolone as assessed by measurements of RNFLT-G and low-contrast visual acuity in the affected eye 6 months after randomisation. Inclusion criteria are a first episode of optic neuritis with decreased visual acuity to  $\leq 0.5$  (decimal system) and an onset of symptoms within 10 days prior to inclusion. The most important exclusion criteria are history of optic neuritis or multiple sclerosis or any ocular disease (affected or non-affected eye), significant hyperopia, myopia or astigmatism, elevated blood pressure, thrombotic events or malignancy. After randomisation, patients either receive 33 000 international units human recombinant erythropoietin intravenously for 3 consecutive days or placebo (0.9% saline) administered intravenously. With an estimated power of 80%, the calculated sample size is 100 patients. The trial started in September 2014 with a planned recruitment period of 30 months.

**Ethics and dissemination:** TONE has been approved by the Central Ethics Commission in Freiburg (194/14)

## Strengths and limitations of this study

- This study examines a potentially neuroprotective agent in optic neuritis, a disease with clearly defined kinetics of neurodegeneration.
- We assess potential neuroprotective effects of erythropoietin by combining a multitude of complementary morphological and functional measures.
- Retinal nerve fibre layer degeneration correlates with general brain atrophy in multiple sclerosis; therefore, the results of the study are meaningful for multiple sclerosis in general.
- The dosage of erythropoietin was chosen according to a prior phase II trial without having performed a proper dose-finding study.
- In order to start treatment as soon as possible, we do not distinguish between possible subtypes of optic neuritis at the time of inclusion.

and the German Federal Institute for Drugs and Medical Devices (61-3910-4039831). It complies with the Declaration of Helsinki, local laws and ICH-GCP.

**Trial registration number:** NCT01962571.

## INTRODUCTION

### Background and trial rationale

Optic neuritis (ON) is one of the most common manifestations of multiple sclerosis (MS) and leads to neurodegeneration in the optic nerve and the retina causing persistent visual impairment. Methylprednisolone pulse therapy is the standard treatment for acute



ON. Although it accelerates visual recovery, it does not influence visual outcome, lesion length or atrophy of the optic nerve.<sup>1</sup> In an animal model of ON, methylprednisolone even increased retinal ganglion cell degeneration by inhibition of a neurotrophin-dependent pathway.<sup>2</sup> Data from animal models of ON indicate that the downstream mechanisms of neurodegeneration involve pathways regulated by neurotrophins.<sup>3</sup> Erythropoietin (EPO) has shown neurotrophin-like properties in models of ischaemia, trauma, epilepsy and MS.<sup>4</sup> In contrast to 'classical' neurotrophins, EPO can be administered systemically. In an animal model of ON, EPO was particularly effective when given in combination with methylprednisolone.<sup>5</sup> Encouraged by these results, a phase II pilot trial was conducted in patients presenting with ON as a first clinical event indicative of MS.<sup>6</sup> In the EPO treatment group, a significant protection of the retinal nerve fibre layer (RNFL) was determined by optical coherence tomography (OCT).

### Clinical evidence for optic nerve neuroprotection by EPO

Diem *et al*<sup>6</sup> have performed a pilot randomised clinical trial in preparation to this phase III trial (VISION PROTECT, NCT00355095): 40 patients were assigned to receive either 33 000 international units (IU) EPO or placebo intravenous injections per day for 3 days in addition to methylprednisolone pulse therapy. Safety monitoring revealed no specific issues. Thirty-seven patients (20/17 EPO/placebo) were analysed for the primary end point change in retinal nerve fibre layer thickness (RNFLT) according to the intention-to-treat analysis. In patients treated with EPO, RNFLT decreased by a mean of 7.5 µm at week 16 compared to a mean of 16.0 µm in the placebo group ( $p=0.03$ ), measured with time-domain OCT. Decrease in retrobulbar diameter of the optic nerve was smaller in the EPO group ( $p=0.01$ ), determined by MRI. Testing of visual functions revealed trends towards an improved outcome after EPO treatment. The trial regimen chosen in TONE has been adapted from VISION PROTECT, which revealed that the general design is feasible and the chosen outcome measures are adequate.

### Risk-benefit assessment

In general, EPO will not be administered beyond 3 days because neuronal damage in ON is considered to be an acute event. In a schizophrenia trial, 40 000 IU of EPO were given weekly over 3 months resulting in a need for bloodlettings in 8 of 39 patients.<sup>7</sup> In contrast, the treatment over 3 days in the pilot trial VISION PROTECT did not lead to any clinically relevant increases in red blood cell counts.<sup>6</sup>

The majority of recorded adverse events in our pilot study<sup>6</sup> consisted of side effects associated with methylprednisolone such as hot flushes, facial flushing, mood changes or hyperglycaemia and did not occur more frequently in EPO-treated patients. Five of 40 patients complained of headache during the treatment period, 4 of

whom had received EPO. Four serious adverse events were recorded throughout the study but were judged as unrelated to the study medication. Haemoglobin values showed a transient slight increase in the EPO-treated group after 1 week. Blood pressure after treatment with EPO remained stable during the treatment period and did not differ from values after methylprednisolone administration alone. Possible additional side effects—although not reported in the pilot study—include increases in red blood cell and thrombocyte counts, elevations of blood pressure and thromboembolic complications.<sup>8</sup> Since the risk of these events might especially be increased if EPO is combined with methylprednisolone, we exclude patients with vascular risk factors. Other exclusion criteria of our study such as history of malignancy or epilepsy are also explained by the spectrum of undesired effects of EPO.<sup>8</sup> Professional or semi-professional sports activities belong to the exclusion criteria because EPO has been misused for doping, especially to enhance endurance of elite cyclists.<sup>9</sup> Additionally, pure red cell aplasia has been described as a rare but potentially fatal side effect of EPO. It is caused by neutralising antibodies induced by repetitive exposure to subcutaneous but eventually also intravenous EPO formulations.<sup>10</sup> For this reason, we included monitoring of antibodies against EPO into our safety assessment.

## METHODS AND ANALYSIS

### Study design

The study is designed as a prospective, double-blind, randomised controlled trial with participating departments located at German University Medical Centers. The trial has two parallel arms, one with an injection of 33 000 IU human recombinant EPO per day for 3 consecutive days, and the other with placebo administered in an equal fashion. In both arms, patients will receive 1000 mg of methylprednisolone intravenously per day for 3 days according to the standard of care.<sup>11</sup> A 1:1 allocation ratio is applied to ensure equally sized treatment groups. The trial is registered at <http://www.clinicaltrials.gov> (NCT01962571) and <http://www.germanctr.de> (DRKS00005298).

### Primary objective

Determination of the efficacy of EPO compared to placebo given as add-on to methylprednisolone (standard of care) as assessed by measurements of global RNFLT-G and low-contrast visual acuity (LCVA) in the affected eye 6 months after randomisation.

### Setting

Participating trial sites are the University Medical Centers in Berlin, Bonn, Düsseldorf, Erlangen, Essen, Freiburg, Göttingen, Hamburg, Hannover, Heidelberg, Mainz, Munich (LMU/TUM) and Tübingen.

## Population

Patients with acute ON are going to be enrolled in this trial. For diagnosis of ON, the recent consensus diagnostic flow chart published by Petzold *et al*<sup>12</sup> is followed. Patients are only eligible if they provide written informed consent and if the investigator has verified that they meet all of the inclusion criteria and none of the exclusion criteria. Patients of both genders will be enrolled as the results of the preclinical and clinical studies did not indicate any gender effect on the trial treatment in terms of efficacy and safety. Inclusion and exclusion criteria are shown in [box 1](#).

## Treatment

### Experimental intervention

A total of 33 000 IU human recombinant EPO given as a bolus injection (epoetin alfa, Hexal AG, Holzkirchen, Germany) per day intravenously on days 1, 2 and 3 in addition to standard methylprednisolone (1000 mg/day intravenously on days 1, 2 and 3).

### Control intervention

Placebo (0.9% saline) administered intravenously on days 1, 2 and 3 in addition to standard methylprednisolone (1000 mg/day intravenously on days 1, 2 and 3).

## Visit schedule

Following randomisation and initiation of trial treatment (visit 1), the patient should be admitted to the ward at the trial site from days 1 to 3. If this is not possible for logistical reasons, assessments may be carried out on an outpatient basis at the discretion of the investigator. The following visits at weeks 1, 4, 16 and 26 and month 24 will be performed in the outpatient setting. [Table 1](#) shows the schedule of visits. In case of pregnancy, regular follow-up visits will be performed if possible. If pregnancy occurs during the first 3 months after study medication, the treating gynaecologist will be informed to decide about an intensified pregnancy observation.

## Outcome measures

### Primary end point

TONE has two primary end points, which will be tested in a hierarchical manner. The first is the mean (global) RNFLT of all retinal segments along a circle of 12°, that is, 3.5 mm diameter (RNFLT-G-12) of the fellow eye at baseline minus RNFLT-G-12 of the affected eye 6 months after randomisation.<sup>13</sup> RNFLT became a standard measure for neurodegeneration over the recent years.<sup>13 14</sup> Using time-domain OCT, thinning of the RNFL from 100 to 78 µm (means,  $p=0.0001$ ) was seen in the majority of ON patients (74%) within 6 months.<sup>15</sup> RNFLT decline reaches a plateau around 4 months.<sup>13</sup> Several investigations have established that RNFLT measurement by OCT is a robust tool for the quantification of optic nerve axons and the degree of optic nerve atrophy, especially after ON.<sup>16–19</sup> The recent introduction of the spectral-domain OCT technique allows for

more precise quantification of the RNFL, in particular using the Spectralis system which incorporates eye tracking for better retest variability (variation coefficient of 0.6%<sup>20</sup> compared with 5% using time-domain OCT).<sup>21</sup> RNFLT will be determined along a ring of 3.5 mm (approximately 12°) diameter concentrically centred on the Bruch's membrane opening at the optic disc using the Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany). RNFLT has been employed as a primary outcome measure in VISION PROTECT and in ongoing trials NCT01451593 and NCT01073813 and has been recommended as such in the literature.<sup>13 17</sup>

The second primary outcome measure is LCVA in the affected eye 6 months after randomisation. While high-contrast visual acuity (HCVA) recovers well after ON,<sup>11</sup> LCVA is most likely to remain subnormal: 22% of cases with fully recovered HCVA revealed subnormal LCVA.<sup>22</sup> LCVA correlates well with health-related quality of life<sup>23</sup> and correlates significantly with RNFLT<sup>24</sup> in patients with MS after previous ON. LCVA will be recorded by 2.5% low-contrast Sloan letter charts.<sup>24</sup>

### Secondary objectives and end points

In addition to the primary objective, seven OCT analyses will be performed to increase the likelihood of capturing neuroprotective properties:

1. RNFLT in the papillomacular bundle (RNFLT-PMB-12) fellow eye at baseline minus RNFLT-PMB-12 of the affected eye 6 months after randomisation;
2. RNFLT in the temporal quadrant (RNFLT-T-12) fellow eye at baseline minus RNFLT-T-12 of the affected eye 6 months after randomisation;
3. RNFLT-G-12 of the affected eye 6 months after randomisation;
4. RNFLT-PMB-12 of the affected eye 6 months after randomisation;
5. RNFLT-T-12 of the affected eye 6 months after randomisation;
6. Total macular volume (TMV) in the fellow eye at baseline minus TMV of the affected eye 6 months after randomisation;
7. TMV of the affected eye 6 months after randomisation.

In addition to these standardised OCT parameters, the Bern Photographic Reading Center will perform further analyses of manually segmented retinal layers (eg, thickness of RNFL plus inner nuclear layer).

The functional end points HCVA and contrast sensitivity will be assessed using standard Early Treatment Diabetic Retinopathy Study charts (ETDRS charts) and Mars charts, respectively.<sup>25</sup> The visual field will be recorded on automated, static perimeters (Octopus 900 perimeter, Haag-Streit, Köniz, Switzerland) using the newly developed German Adaptive Threshold Estimation (GATE) strategy within the entire 30° visual field in a (fast) threshold determining manner<sup>26</sup> and

**Box 1** Inclusion and exclusion criteria

## Inclusion criteria

1. Written informed consent obtained according to international guidelines and local laws
2. Male and female patients aged  $\geq 18$  to  $\leq 50$  years
3. Patients with optic neuritis (ON)
4. First symptoms of ON  $\leq 10$  days prior to the first administration of investigational product
5. High-contrast visual acuity (HCVA) of 0.5 (decimal system) corresponding to a minimum angle of resolution (MAR) of 2.0, a logMAR value of 0.3, or a Snellen equivalent of 6/12 (UK notation) and 20/40 (UK notation)<sup>12</sup>
6. Adequate optical coherence tomography (OCT) measurements available (according to quality control by Bern Photographic Reading Center)

## Exclusion criteria

1. Patient without legal capacity who is unable to understand the nature, significance and consequences of the trial
2. Simultaneous participation in another interventional trial which could interfere with this trial and/or participation in a clinical trial within the last 3 months before enrolment in this trial
3. Refractive anomalies: hyperopia  $>5$  dpt, myopia  $<-7$  dpt, astigmatism  $>3$  dpt
4. Media opacity
5. Severe papillitis
6. Previous ON
7. Any other optic nerve and retinal disease
8. Pre-existing multiple sclerosis (MS) diagnosed according to the 2005 or 2010 McDonald criteria,<sup>35 36</sup> or any other neurological disease
9. Congenital diseases (thrombophilia, phenylketonuria)
10. Acquired diseases (autoimmune diseases, cardiovascular diseases, diabetes mellitus, uncontrolled hypertension, any malignancy, epilepsy, known tuberculosis with ongoing or unknown activity, acute gastrointestinal ulceration within the past 3 months prior to randomisation, acute viral, bacterial or fungal infection, known infection with HIV, hepatitis B virus, or hepatitis C virus, history of colitis ulcerosa, diverticulitis, or acute enteroanastomosis, known osteoporosis, history of thromboembolic events, elevated haemoglobin level, polycythaemia, any other significant illness potentially interfering with any trial assessment or trial treatment)
11. Performing semiprofessional or professional sporting activities or physical training
12. Pretreatment with corticosteroids in the last 30 days prior to the onset of optic neuritis
13. Pretreatment with erythropoietin (EPO)
14. Known or persistent abuse of medication, drugs or alcohol
15. Active immunisation within 2 weeks prior to randomisation
16. Significant surgery within 4 weeks prior to randomisation
17. Blood donation or bloodletting within 4 weeks prior to screening
18. Pretreatment with immunosuppressive or immunomodulatory agents
19. Persons who are in a relationship of dependence/employment with the sponsor or the investigator
20. Female patients: pregnancy, planned pregnancy within the next 3 months or lactation period
21. Female patients: inability or unwillingness to use two effective methods of contraception (barrier methods, hormonal methods or abstinence) during the initial 3 months of the study

analysed centrally by the Center of Competence 'Vision Research' of the Aalen University, Germany. Neurological examinations will be performed at each visit to assess the Extended Disability Status Score (EDSS), an international standard neurological symptom severity classification system. Amplitudes and latencies of visual evoked potentials will be measured by standard electrophysiological equipment in accordance with the recommendation of the International Society for Clinical Electrophysiology. Quality of life will be assessed using the validated German version of 25-item National Eye Institute Visual Functioning Questionnaire (Interviewer Version).<sup>27</sup> Its overall score will be analysed. This questionnaire proved to be sensitive to chronic eye disease including ON.<sup>23 24</sup>

In addition, relapse rates of ON and/or MS will be documented and analysed. Safety will be evaluated in terms of adverse events, serious adverse events and laboratory data (haemoglobin, EPO antibodies).

**Sample size**

Sample size calculations are based on RNFLT-G-12. For the second primary end point LCVA, power calculations with the resulting sample size have been performed. The primary analysis will be performed applying a linear regression model for the primary end point (RNFLT-G-12 of the contralateral healthy eye at baseline minus RNFLT-G-12 in the affected eye 6 months after randomisation) with RNFLT-G-12 of the contralateral healthy eye at baseline as a covariate. The resulting treatment estimate is identical to the treatment estimate resulting from a linear regression model with the same covariates, but using RNFLT-G-12 in the affected eye 6 months after randomisation instead as the dependent variable. Therefore, the following calculations based on the end point RNFLT-G-12 in the affected eye 6 months after randomisation are valid considerations for the required sample size. The sample size revision planned after 50 patients will use the SD of the primary end point.

**Table 1** Visit schedule

| Action                                      | Screening/<br>baseline day 0<br>(-3 days) | Therapy day<br>1        | Therapy day<br>2        | Therapy day<br>3        | Week 1<br>(±4 days) | Week 4<br>(±4 days) | Week 16<br>(±4 days) | Week 26<br>(±7 days) | Month 24<br>(±7 days) |
|---|---|-------------------------|-------------------------|-------------------------|---------------------|---------------------|----------------------|----------------------|-----------------------|
| Informed consent                            | X   |                         |                         |                         |                     |                     |                      |                      |                       |
| Demographics                                | X   |                         |                         |                         |                     |                     |                      |                      |                       |
| Inclusion/exclusion criteria                | X   |                         |                         |                         |                     |                     |                      |                      |                       |
| Medical history                             | X   |                         |                         |                         |                     |                     |                      |                      |                       |
| Present complaints                          | X   | X                       | X                       | X                       | X                   | X                   | X                    | X                    | X                     |
| MS relapse/ON<br>recurrence                 |   |                         |                         |                         |                     | X                   | X                    | X                    | X                     |
| Concomitant medication                      | X   | X                       | X                       | X                       | X                   | X                   | X                    | X                    | X                     |
| Physical examination                        | X*  |                         |                         |                         | X*                  | X*                  |                      |                      |                       |
| Vital signs†                                | X   | 3 X                     | 3 X                     | 3 X                     | X                   | X                   |                      |                      |                       |
| Body weight                                 | X*  |                         |                         |                         | X*                  | X*                  |                      |                      |                       |
| ECG   | X*  |                         |                         |                         | X*                  |                     |                      |                      |                       |
| Routine laboratory‡                         | X   |                         |                         |                         | X                   | X                   |                      | X                    |                       |
| Urinalysis (if clinically<br>indicated)     | X*  |                         |                         |                         | (X*)                | (X*)                | (X*)                 | (X*)                 |                       |
| Pregnancy test                              | X*  |                         |                         |                         |                     |                     |                      |                      |                       |
| EPO antibodies (analysis<br>in central lab) | X   |                         |                         |                         |                     |                     |                      | X                    |                       |
| Aquaporin 4 antibodies                      | X   |                         |                         |                         |                     |                     |                      |                      |                       |
| Methylprednisolone<br>therapy               |   | X (standard<br>of care) | X (standard<br>of care) | X (standard<br>of care) |                     |                     |                      |                      |                       |
| EPO/placebo<br>administration               |   | X                       | X                       | X                       |                     |                     |                      |                      |                       |
| AE reporting                                | X   | X                       | X                       | X                       | X                   | X                   | X                    | X                    | X§                    |
| Neurological examination,<br>EDSS¶          | X   |                         |                         |                         | X                   | X                   | X                    | X                    | X                     |
| Randomisation                               | X   |                         |                         |                         |                     |                     |                      |                      |                       |
| Refraction                                  | X   |                         |                         |                         | X                   | X                   | X                    | X                    | X                     |
| OCT   | X   |                         |                         |                         |                     | X                   | X                    | X                    | X                     |
| LCVA  | X   |                         |                         |                         | X                   | X                   | X                    | X                    | X                     |
| HCVA  | X   |                         |                         |                         | X                   | X                   | X                    | X                    | X                     |

Continued



Table 1 Continued

| Action      | Screening/<br>baseline day 0<br>(-3 days) | Therapy day<br>1 | Therapy day<br>2 | Therapy day<br>3 | Therapy day<br>Week 1<br>(±4 days) | Week 4<br>(±4 days) | Week 16<br>(±4 days) | Week 26<br>(±7 days) | Month 24<br>(±7 days) |
|-------------|---|------------------|------------------|------------------|------------------------------------|---------------------|----------------------|----------------------|-----------------------|
| CS          | X   |                  |                  |                  | X                                  | X                   | X                    | X                    | X                     |
| Perimetry   | X   |                  |                  |                  | X                                  | X                   | X                    | X                    | X                     |
| NEI VFQ-25  | X   |                  |                  |                  |                                    |                     | X                    | X                    | X                     |
| VEP         | X   |                  |                  |                  |                                    | X                   |                      | X                    | X                     |
| Routine MRI | X**                                       |                  |                  |                  |                                    |                     |                      | X††                  | (X)                   |

\*Test to be performed, but not recorded on CRF.  
†CRF: only blood pressure.  
‡CRF: only haemoglobin (Hb).  
§(S)/AE: to be reported only if related to investigational product.  
¶CRF: only EDSS.  
\*\*Can be done after baseline as per local routine.  
††Recommended.  
AE, adverse event; CS, contrast sensitivity; EDSS, Extended Disability Status Score; EPO, erythropoietin; HCVA, high-contrast visual acuity; LCVA, low-contrast visual acuity; MS, multiple sclerosis; NEI VFQ-25, National Eye Institute Visual Functioning Questionnaire 25; OCT, optical coherence tomography; ON, optic neuritis; VEP, visual evoked potentials.

### Sample size calculation for RNFLT-G-12

In order to determine the required sample size at a given significance level  $\alpha$  by means of a two-sided t test, it is required to specify the clinically relevant difference between treatment groups for which a power of 80% is necessary, and assumptions on the SD of the primary end point. We have based these assumptions on the available studies published so far. For healthy or unaffected fellow eyes, the RNFLT-G-12 ranges around 100  $\mu\text{m}$  with a SD of less than 15  $\mu\text{m}$ .<sup>13 16 28–31</sup> After ON, the following data have been published (RNFLT-G-12 in micrometre with mean $\pm$ SD, follow-up interval): 68.7  $\pm$ 18.8, 3 years after clinically isolated syndrome (CIS);<sup>29</sup> 85 $\pm$ 17, >1 month after MS;<sup>16</sup> 65 $\pm$ 17; <6 months after CIS;<sup>30</sup> 84 $\pm$ 12, 12 months after CIS<sup>31</sup> and 83 $\pm$ 18, 6 months after CIS.<sup>13</sup> In the ON model, EPO reduced retinal ganglion cell loss by 50%.<sup>5</sup> Adapted to the RNFLT-G-12, this effect would result in about 10  $\mu\text{m}$  RNFLT-G-12 difference which is in exact agreement with the effect size observed in VISION PROTECT.<sup>6</sup> These investigations are the basis of our assumptions: For patients with ON, a thinning of around 20% (15% to 25%) can be assumed in the placebo group. The absolute values in the cited studies ranged from 11 to 26  $\mu\text{m}$ . In view of this range, a difference of 10  $\mu\text{m}$  between treatment groups is considered to be clinically relevant. Our sample size calculation is based on the assumptions mentioned above with an SD of 17  $\mu\text{m}$  and a clinically relevant difference between treatment groups ( $\delta$ ) of 10  $\mu\text{m}$  with an  $\alpha$  of 5% and a power of 80%. No adjustment for multiple testing (two primary end points) is necessary, as we are applying a hierarchical testing procedure. The resulting sample size is 47 per group and 94 patients in total, based on the two-sided t test. Since the possibility of a proportion (5%) of non-evaluable patients must be considered, 100 patients will be randomised.

### Sensitivity analysis of sample size calculation for RNFLT-G-12

In the primary analysis, a regression model adjusting for baseline RNFLT-G-12 of the fellow eye and stratifying factors (eg, study site) will be applied. Less variability and hence higher power can then be expected compared with the usual calculation via t test. Using the two-sided t test for the sample size calculation therefore provides a conservative strategy. Table 2 provides sample size calculations for different scenarios.

Table 2 displays resulting sample sizes per group depending on different  $\delta$ , SDs and power values. First, we considered our assumptions of an expected difference of 10  $\mu\text{m}$  with an SD of 17  $\mu\text{m}$  as reasonable and calculated the power depending on the sample size (n, left table). Next, we varied the assumptions concerning the difference between treatment groups (middle table) and the SD assumptions (right table). Sample sizes per group are listed and denoted as n.

**Table 2** Sample size calculations for different scenarios

| n   | $\delta$ | SD | $\alpha$ | Power | n  | $\delta$ | SD | $\alpha$ | Power | n  | $\delta$ | SD | $\alpha$ | Power |
|-----|----------|----|----------|-------|----|----------|----|----------|-------|----|----------|----|----------|-------|
| 20  | 10       | 17 | 0.05     | 0.442 | 72 | 8        | 17 | 0.05     | 0.801 | 28 | 10       | 13 | 0.05     | 0.807 |
| 30  | 10       | 17 | 0.05     | 0.610 | 47 | 10       | 17 | 0.05     | 0.806 | 32 | 10       | 14 | 0.05     | 0.803 |
| 40  | 10       | 17 | 0.05     | 0.738 | 33 | 12       | 17 | 0.05     | 0.806 | 37 | 10       | 15 | 0.05     | 0.808 |
| 50  | 10       | 17 | 0.05     | 0.830 | 25 | 14       | 17 | 0.05     | 0.814 | 42 | 10       | 16 | 0.05     | 0.808 |
| 60  | 10       | 17 | 0.05     | 0.892 | 19 | 16       | 17 | 0.05     | 0.806 | 47 | 10       | 17 | 0.05     | 0.806 |
| 70  | 10       | 17 | 0.05     | 0.933 | 16 | 18       | 17 | 0.05     | 0.826 | 52 | 10       | 18 | 0.05     | 0.801 |
| 80  | 10       | 17 | 0.05     | 0.959 | 13 | 20       | 17 | 0.05     | 0.821 | 58 | 10       | 19 | 0.05     | 0.802 |
| 90  | 10       | 17 | 0.05     | 0.975 | 11 | 22       | 17 | 0.05     | 0.823 | 64 | 10       | 20 | 0.05     | 0.801 |
| 100 | 10       | 17 | 0.05     | 0.985 | 9  | 24       | 17 | 0.05     | 0.803 | 71 | 10       | 21 | 0.05     | 0.804 |

Sample size calculations by other authors have yielded  $n=100$ <sup>32</sup> and  $n=116$  considering only the affected eyes and  $n=72$  considering the difference in RNFLT-G-12 between follow-up of the affected eye and baseline of the fellow eye.<sup>13</sup> All such calculations are based on time-domain OCT data. Spectral-domain OCT, as used in this trial with higher spatial resolution, is likely to provide greater accuracy.

When the primary end point measurements of 50 patients are obtained, a blinded estimate of the SD will be performed (total variance ignoring treatment assignment).<sup>33</sup> A revised sample size calculation based on the new SD estimation will be obtained. No practically relevant differences on the nominal significance level have been observed in the investigations of this procedure.<sup>33</sup>

#### Power calculations for LCVA

For LCVA as the other primary end point, less evidence from published data is available so far. However, sample size and power calculations have been performed for LCVA in analogy to RNFLT-G-12. In patients with MS and previous ON, mean LCVA >3 months after onset of ON was 11.4 letters (SD 10.6) compared with 18.0 (SD 9.6) in MS non-ON eyes and 24.8 (SD 7.4) in healthy controls.<sup>24</sup> Median LCVA values of 15 in MS ON eyes, 24 in MS non-ON eyes and 32 in healthy controls were reported in another publication.<sup>16</sup> With 47 patients per arm, 80% power is achieved with an assumed SD of 10.6 for a group difference of 6.2, corresponding to an expected LCVA of  $11.4+6.2=17.6$  in the EPO arm. Similarly, 47 patients per arm provide 70% power for  $\delta$  of 5.5 and expected LCVA of 16.9 in the EPO arm. Therefore, the proposed sample size based on RNFLT-G-12 provides sufficient power for the second primary outcome as well.

#### Randomisation and blinding

##### Blinding

The study medication will be prepared in a non-blinded fashion by the pharmacy staff. The investigator who will remain blinded to the patient's assigned treatment group will forward a copy of the completed randomisation form to the pharmacist. By means of a randomisation list provided by the sponsor, the pharmacist will

prepare the investigational product (verum or placebo) according to the randomisation algorithm and will document the corresponding patient number.

#### Randomisation methodology

To allow for flexible randomisation independent of office hours, randomisation will be performed by using sealed envelopes provided by the sponsor, which are kept at the clinical trial sites. The randomisation code will be generated by the Clinical Trials Unit, Medical Center—University of Freiburg. Randomisation will be performed stratified by trial site, in blocks of variable size in a ratio of 1:1. Block size will be documented separately and will not be disclosed to the sites. The randomisation code will be produced by validated programmes based on the Statistical Analysis System (SAS).

#### Statistical analysis

A detailed statistical analysis plan will be prepared. A blind review of the data will be performed after the end of the planned follow-up period without looking at the randomised treatment for each patient. If the statistical analysis plan contains any changes to the analyses outlined in the trial protocol, they will be marked as such, and reasons for amendments will be given. All statistical programming for analysis will be performed with the SAS.

#### Definition of populations included in the analyses

The primary efficacy analysis will be conducted according to the intention-to-treat principle and will therefore be based on the full analysis set. It preserves the treatment allocation provided by randomisation and will be as close as possible to the intention-to-treat ideal of including all randomised patients. Patients without any postrandomisation data will be excluded from the full analysis set. For patients with missing data for the primary end points, the last available outcome measurement will be used for the calculation of the primary end point.

The per-protocol population is a subset of the full analysis set and is defined as the group of patients who underwent the examinations required for the assessment



of the end points at all times. Efficacy analyses will be performed in the per-protocol population for a sensitivity analysis. During the blind review of the data, it will be decided which patients belong to the per-protocol population.

Safety analyses will be performed in the safety population. Patients in the safety population are analysed as belonging to the treatment arm defined by treatment received. Patients are included in the respective treatment arm, if they received at least one dose of trial treatment.

### Primary end point

As this study has two primary end points, a hierarchical testing procedure will be applied in order to preserve the type I error  $\alpha=5\%$ . RNFLT-G-12 will be tested first. Only in case a significant difference between treatment groups can be demonstrated, the test procedure for the end point LCVA will be carried out. To formalise the statistical approach, the following notation is used:

$\tau_{\text{EPO}} - \tau_{\text{PLA}}$ : RNFLT-G-12 difference after 6 months between EPO group and placebo group

$\theta_{\text{EPO}} - \theta_{\text{PLA}}$ : LCVA difference after 6 months between EPO group and placebo group.

The following two null hypotheses are tested hierarchically at 5% level:

1.  $H_0: \tau_{\text{EPO}} - \tau_{\text{PLA}} = 0$  vs  $H_1: \tau_{\text{EPO}} - \tau_{\text{PLA}} \neq 0$ ,
2.  $H_0: \theta_{\text{EPO}} - \theta_{\text{PLA}} = 0$  vs  $H_1: \theta_{\text{EPO}} - \theta_{\text{PLA}} \neq 0$ .

For both primary end points, the hypothesis will be tested within a linear regression model, using the outcome after 26 weeks as the dependent variable, and with treatment assignment, study site and baseline measurement (RNFLT-G-12 and LCVA, respectively) constituting independent variables (CPMP/EWP/2863/99). RNFLT baseline measurements of the affected eye will be subject to additional variation caused by swelling of the optic disc. Therefore, baseline RNFLT-G-12 measurements of the fellow eye will be used instead, as they are an adequate covariate to reduce variation of the treatment effect estimate. The definition of the first primary end point in the linear regression model is RNFLT-G-12 of the contralateral healthy eye at baseline minus RNFLT-G-12 in the affected eye 6 months after randomisation. The resulting treatment estimate from this model is identical (apart from the sign) to the treatment estimate resulting from a linear regression model with the same covariates, but using RNFLT-G-12 in the affected eye 6 months after randomisation instead as the dependent variable. Within the linear regression model, the treatment effect will be estimated and presented with a two-sided 95% CI, and a two-sided test of the treatment difference will be performed at the 5% significance level.

For patients with missing data for the primary end points, the last available outcome measurement will be used for the calculation of the primary end point. This last-observation-carried-forward strategy seems reasonable, as RNFLT deteriorates in the course of time and

95% of the expected RNFLT loss occurs at a mean of 3 months after the onset of symptoms.<sup>13</sup> Rates of adverse events leading to early termination of treatment are expected to be very low because study medication will only be given from days 1 to 3 of the trial. VISION PROTECT had no drop-outs due to adverse events or serious adverse events.<sup>6</sup> As observations after 3 months can be expected to be similar to observations after 6 months, it can be assumed that this procedure does not notably affect variance estimation. Sensitivity analyses will be conducted by using multiple imputation methods. The censoring distributions (time to drop-out) will be compared for the randomised treatment groups. Reasons for drop-out will be explored.

### Secondary end points of efficacy

The secondary end points will be analysed as the dependent variable in appropriate regression models. MS and ON relapse rates will be estimated by the Kaplan-Meier method, and Cox proportional hazards regression models will be applied.

### Safety parameters

All adverse events will be listed by trial site and patient and displayed in summary tables. The total number of adverse events, the minimum, maximum and mean number of adverse events per patient, the total number of follow-up days (number of days in the observation period), the number of adverse events per follow-up day (total number of adverse events divided by the total by the number of follow-up days), the number of patients who had at least one adverse event and the number of patients who stopped treatment due to adverse events will be given.

### Interim analysis

An interim analysis is planned for the time point when 50 patients have been randomised and followed up for 6 months. The interim report will describe patient recruitment, treatment compliance as well as safety for the patients in this period. Only blinded analyses will be conducted in the interim analysis, since no major safety problems are expected.

Additionally, a blinded estimate of the SD of the primary end point will be calculated (total variance ignoring treatment assignment).<sup>33</sup> A revised sample size calculation based on the new SD estimation will be performed. The results of the interim analysis will be reported only to the independent data monitoring committee (DMC). The DMC will give advice to the coordinating investigators concerning the further conduct of the trial.

### Data monitoring committee

An independent DMC has been established. The function of the DMC is to monitor the course of the study and if necessary to give a recommendation to the coordinating investigator and sponsor of the trial for discontinuation, modification or continuation of the study.

Furthermore, the DMC may assist in the preparation of Development Safety Update Reports.

### Study progress

The recruitment period had started in September 2014 with opening of first study centre. In March 2015, the last of the currently running 13 study centres was initialised. As of 5 February 2016, we have included a total of 51 patients.

### DISCUSSION

ON is currently not effectively treated because immunotherapies available so far have only limited neuroprotective properties. ON offers several advantages for testing neuroprotective agents as it represents a homogenous disease with rapid and predictable neurodegeneration. OCT allows for imaging of the proximal axon fibres of retinal ganglion cells, a subpopulation of central nervous system neurons, without the presence of confounding myelin sheaths. Additionally, visual dysfunction during and after ON can be precisely characterised by functional and electrophysiological measurements. This interdisciplinary study represents a conjoined effort of neurologists and ophthalmologists and is designed as a prospective, double-blind randomised clinical trial conducted in different German University Medical Centers. It substantiates a recent pilot trial by R Diem, which showed a neuroprotective effect for EPO in ON.<sup>6</sup> A positive outcome of TONE would not only improve the treatment for ON but also have implications on therapy development in MS, since RNFL atrophy is a surrogate marker for neuronal degeneration in MS<sup>16</sup> and correlates with general brain atrophy.<sup>34</sup>

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### REFERENCES

1. Gal RL, Vedula SS, Beck R. Corticosteroids for treating optic neuritis. *Cochrane Database Syst Rev* 2015;8:CD001430.
2. Diem R, Hobom M, Maier K, *et al.* Methylprednisolone increases neuronal apoptosis during autoimmune CNS inflammation by inhibition of an endogenous neuroprotective pathway. *J Neurosci* 2003;23:6993–7000.
3. Sättler MB, Merkler D, Maier K, *et al.* Neuroprotective effects and intracellular signaling pathways of erythropoietin in a rat model of multiple sclerosis. *Cell Death Differ* 2004;11:S181–92.
4. Brines ML, Ghezzi P, Keenan S, *et al.* Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. *Proc Natl Acad Sci U S A* 2000;97:10526–31.
5. Diem R, Sättler MB, Merkler D, *et al.* Combined therapy with methylprednisolone and erythropoietin in a model of multiple sclerosis. *Brain* 2005;128:375–85.
6. Sühs KW, Hein K, Sättler MB, *et al.* A randomized, double-blind, phase 2 study of erythropoietin in optic neuritis. *Ann Neurol* 2012;72:199–210.



7. Ehrenreich H, Hinze-Selch D, Stawicki S, *et al*. Improvement of cognitive functions in chronic schizophrenic patients by recombinant human erythropoietin. *Mol Psychiatry* 2007;12:206–20.
8. Sirén AL, Fasshauer T, Bartels C, *et al*. Therapeutic potential of erythropoietin and its structural or functional variants in the nervous system. *Neurotherapeutics* 2009;6:108–27.
9. Heuberger JA, Cohen Tervaert JM, Schepers FM, *et al*. Erythropoietin doping in cycling: lack of evidence for efficacy and a negative risk-benefit. *Br J Clin Pharmacol* 2013;75:1406–21.
10. Pollock C, Johnson DW, Hörl WH, *et al*. Pure red cell aplasia induced by erythropoiesis-stimulating agents. *Clin J Am Soc Nephrol* 2008;3:193–9.
11. Beck RW, Cleary PA, Anderson MM Jr, *et al*. A randomized, controlled trial of corticosteroids in the treatment of acute optic neuritis. The Optic Neuritis Study Group. *N Engl J Med* 1992;326:581–8.
12. Petzold A, Wattjes MP, Costello F, *et al*. The investigation of acute optic neuritis: a review and proposed protocol. *Nat Rev Neurol* 2014;10:447–58.
13. Henderson AP, Altmann DR, Trip AS, *et al*. A serial study of retinal changes following optic neuritis with sample size estimates for acute neuroprotection trials. *Brain* 2010;133:2592–602.
14. Petzold A, de Boer JF, Schippling S, *et al*. Optical coherence tomography in multiple sclerosis: a systematic review and meta-analysis. *Lancet Neurol* 2010;9:921–32.
15. Costello F, Coupland S, Hodge W, *et al*. Quantifying axonal loss after optic neuritis with optical coherence tomography. *Ann Neurol* 2006;59:963–9.
16. Fisher JB, Jacobs DA, Markowitz CE, *et al*. Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. *Ophthalmology* 2006;113:324–32.
17. Frohman E, Costello F, Zivadinov R, *et al*. Optical coherence tomography in multiple sclerosis. *Lancet Neurol* 2006;5:853–63.
18. Trip SA, Schlottmann PG, Jones SJ, *et al*. Quantification of optic nerve head topography in optic neuritis: a pilot study. *Br J Ophthalmol* 2006;90:1128–31.
19. Trip SA, Schlottmann PG, Jones SJ, *et al*. Optic nerve atrophy and retinal nerve fibre layer thinning following optic neuritis: evidence that axonal loss is a substrate of MRI-detected atrophy. *Neuroimage* 2006;31:286–93.
20. Menke MN, Dabov S, Knecht P, *et al*. Reproducibility of retinal thickness measurements in healthy subjects using Spectralis optical coherence tomography. *Am J Ophthalmol* 2009;147:467–72.
21. Budenz DL, Fredette MJ, Feuer WJ, *et al*. Reproducibility of peripapillary retinal nerve fiber thickness measurements with stratus OCT in glaucomatous eyes. *Ophthalmology* 2008;115:661–6.
22. Steel DH, Waldock A. Measurement of the retinal nerve fibre layer with scanning laser polarimetry in patients with previous demyelinating optic neuritis. *J Neurol Neurosurg Psychiatry* 1998;64:505–9.
23. Mowry EM, Loguidice MJ, Daniels AB, *et al*. Vision related quality of life in multiple sclerosis: correlation with new measures of low and high contrast letter acuity. *J Neurol Neurosurg Psychiatry* 2009;80:767–72.
24. Walter SD, Ishikawa H, Galetta KM, *et al*. Ganglion cell loss in relation to visual disability in multiple sclerosis. *Ophthalmology* 2012;119:1250–7.
25. Arditi A. Improving the design of the letter contrast sensitivity test. *Invest Ophthalmol Vis Sci* 2005;46:2225–9.
26. Schiefer U, Pätzold J, Dannheim F. Konventionelle Perimetrie. *Ophthalmologie* 2005;102:627–46.
27. Mangione CM, Lee PP, Gutierrez PR, *et al*. Development of the 25-item National Eye Institute Visual Function Questionnaire. *Arch Ophthalmol* 2001;119:1050–8.
28. Bowd C, Zangwill LM, Medeiros FA, *et al*. Structure-function relationships using confocal scanning laser ophthalmoscopy, optical coherence tomography, and scanning laser polarimetry. *Invest Ophthalmol Vis Sci* 2006;47:2889–95.
29. Trip SA, Schlottmann PG, Jones SJ, *et al*. Retinal nerve fiber layer axonal loss and visual dysfunction in optic neuritis. *Ann Neurol* 2005;58:383–91.
30. Wang XL, Yu T, Xia DZ, *et al*. Measurement of retinal nerve fiber layer thickness in optic atrophy eyes of patients with optic neuritis using optical coherence tomography. *Graefes Arch Clin Exp Ophthalmol* 2010;248:1013–18.
31. Klistorner A, Arvind H, Garrick R, *et al*. Interrelationship of optical coherence tomography and multifocal visual-evoked potentials after optic neuritis. *Invest Ophthalmol Vis Sci* 2010;51:2770–7.
32. Kupersmith MJ, Gal RL, Beck RW, *et al*. Visual function at baseline and 1 month in acute optic neuritis: predictors of visual outcome. *Neurology* 2007;69:508–14.
33. Friede T, Kieser M. Sample size recalculation in internal pilot study designs: a review. *Biom J* 2006;48:537–55.
34. Gordon-Lipkin E, Chodkowski B, Reich DS, *et al*. Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis. *Neurology* 2007;69:1603–9.
35. Polman CH, Reingold SC, Edan G, *et al*. Diagnostic criteria for multiple sclerosis: 2005 revisions to the “McDonald Criteria”. *Ann Neurol* 2005;58:840–6.
36. Polman CH, Reingold SC, Banwell B, *et al*. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011;69:292–302.

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## Treatment of optic neuritis with erythropoietin (TONE): a randomised, double-blind, placebo-controlled trial—study protocol

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