Terazosin RepUrposing STudy in ALS (TRUST)

A pilot study targeting PGK1 with terazosin in ALS patients

Internal Reference Number / Short title: TRUST

Ethics Ref: 21/EM/0251

IRAS Project ID: 301752

EudraCT Number: 2021-003345-38

Date and Version No: V2.0, 13Dec2021

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The investigators declare no potential conflicts of interest.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

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1. KEY TRIAL CONTACTS

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2. LAY SUMMARY

Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease (MND), is a complex neurodegenerative disease for which treatment options remain very limited. Although the disease has many causes, the vast majority of patients have evidence of deposition of a protein called TDP-43 in their brains at autopsy, and mutations in the *TARDBP* gene (which encodes TDP-43) are a cause of familial ALS. Therefore, laboratory models of TDP-43 dysfunction are being used to screen drugs of potential benefit in ALS. Evidence has recently emerged suggesting that elevating or activating an enzyme called

phosphoglycerate kinase 1 (PGK1) can protect nerve cells against degeneration by increasing the energy status of the cells. Using high throughput screening of drugs in laboratory models of ALS, Edinburgh researchers, led by Prof Tom Gillingwater (Co-Investigator), have identified terazosin, a known small molecular activator of PGK1, as a drug which potentially reduces TDP-43 toxicity. The Edinburgh and Oxford teams have validated its effects in promoting motor neuron survival across multiple ALS models (mice, zebrafish, primary motor neurons). Terazosin is routinely prescribed to patients with hypertension or symptoms from an enlarged prostate, and is thus an ideal drug for repurposing. However, mounting a full-scale clinical trial to assess efficacy is unrealistic, without more evidence of a biological impact on the disease process, since the drug is off-patent. In the current study, we will test whether terazosin treatment significantly affects key biomarkers of neurodegeneration (neurofilament levels and macrophage markers in CSF) in a cohort of 50 patients with ALS. Although not designed or powered to assess clinical benefit at the individual or group level, if successful, this would provide evidence that terazosin may modify the disease process and should lead to rapid incorporation of the drug into ongoing larger-scale international clinical trials to assess efficacy, using platform designs.

3. SYNOPSIS

Trial title	Terazosin RepUrposing STudy in ALS (TRUST): A pilot study targeting PGK1 with terazosin in ALS patients		
Internal ref. no./short title	TRUST		
Trial registration	EudraCT: 2021-003345-38 ISRCTN: ISRCTN45028842		
Sponsor	University of Oxford Research Governance, Ethics and Assurance Team Joint Research Office 1st floor, Boundary Brook House Churchill Drive, Headington Oxford OX3 7GB		
Funder	My Name'5 Doddie Foundation c/o Gilson Gray LLP 29 Rutland Square Edinburgh EH1 2BW		
Clinical phase	Pilot		
Trial design	A single-centre, interventional, open-label, non-randomised, non-controlled proof of concept study to assess the neuroprotective effects of terazosin in patients with ALS		
Trial participants	Patients with ALS attending the Oxford MND clinic		
Sample size	50 patients		
Planned trial period	Total project duration: 24 months Individual participant's involvement: 6 months		
Planned recruitment period	Recruitment start date: 01Oct2021 Recruitment end date: 31Jul2022		
	Objectives	**Outcome measures**	**Time points**
Primary	To evaluate the futility of terazosin as treatment for ALS using change in plasma neurofilament levels	Proportion of participants with >30% decrease in plasma neurofilament light chain at 6 months, compared with baseline levels	Baseline and 6 months
Secondary	1. To measure change in neurodegeneration, microglial and energy metabolism biomarkers in	1a. Linear models of neurodegeneration and energy metabolism markers: •CSF: neurofilament light chain, phosphorylated neurofilament heavy	Baseline, 3 months and 6 months

	relation to clinical measures and terazosin treatment in patients with ALS 2. To measure the frequency of adverse events in ALS patients treated with terazosin	chain, chitotriosidase 1, chitinase 3-like protein 1, chitinase 3-like protein 2 and PGK1 •Plasma: neurofilament light chain and phosphorylated neurofilament heavy chain •Urine: Titin N-terminal fragment With clinical measures: •Revised ALS Functional Rating Scale (ALSFRS-R) score •Forced Vital Capacity (FVC) •Survival •Cumulative terazosin treatment 1b. Longitudinal changes in biomarker levels comparing baseline with 3 and 6 months 1c. Changes in biomarker and clinical parameter levels in relation to historical control data 2. Adverse events related to IMP and proportion of patients that drop out due to adverse events	
Intervention	IMP: Terazosin 10mg per tablet (oral administration) nIMP: Riluzole, as prescribed by treating clinician		
Comparator	None		

4. ABBREVIATIONS

AE	Adverse Event
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-R	Revised ALS Functional Rating Scale
AR	Adverse Reaction
CHI3L1	Chitinase 3-like protein 1
CHI3L2	Chitinase 3-like protein 2
CHIT1	Chitotriosidase-1
CI	Chief Investigator
CRF	Case Report Form
CSF	Cerebrospinal fluid
CTA	Clinical Trials Authorisation
DSMC	Data & Safety Monitoring Committee
DSUR	Development Safety Update Report
ECAS	Edinburgh Cognitive and Behavioural ALS Screen
eCRF	Electronic Case Report Form
EMA	European Medicines Agency
FBC	Full Blood Count
FDA	Food and Drug Administration
FTD	Frontotemporal Dementia
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GP	General Practitioner

HRA	Health Research Authority
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
LFT	Liver Function Test
MHRA	Medicines and Healthcare products Regulatory Agency
MND	Motor Neuron Disease
NFL	Neurofilament light chain
NHS	National Health Service
NIV	Non-Invasive Ventilation
OCTRU	Oxford Clinical Trials Research Unit
OUH	Oxford University Hospitals
PEG	Percutaneous Endoscopic Gastrostomy
PGK-1	Phosphoglycerate kinase 1
PI	Principal Investigator
PIS	Participant / Patient Information Sheet
pNFH	Phosphorylated Neurofilament heavy chain
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RGEA	Research Governance, Ethics and Assurance Team, University of Oxford
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TDP-43	Transactive response DNA-binding Protein 43
TMF	Trial Master File
TOG	Trial Oversight Group
TSC	Trial Steering Committee
U&E	Urea and electrolytes
WOCBP	Woman of childbearing potential

5. BACKGROUND AND RATIONALE

5.1. Amyotrophic lateral sclerosis (motor neuron disease)

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease affecting the neurological network responsible for voluntary movement. It is uniformly fatal and malignantly progressive, with 10% of patients dying within 12 months of first symptoms(1). Patients present with focal weakness (70% in one limb, 30% in the muscles of speech and swallowing), but loss of function rapidly generalises to affect most areas of the body leading to severe disability(1). Patients die because of respiratory insufficiency, with an overall median survival of 22 months from diagnosis. Familial ALS accounts for up to 10% of all incident cases, and the genetic mutation responsible has now been explained in 70-80% of familial cases, depending on the population studied, with mutations in over twenty different genes, mostly in an autosomal dominant inheritance pattern(2).

An ever-expanding list of genes in which mutation leads to typical ALS have implicated abnormalities in RNA processing, protein homeostasis and axonal transport. How these apparently distinct pathways converge to cause the characteristic clinical syndrome of ALS remains unclear(3). Despite this biological complexity, a unifying feature of ALS is that the majority (>95%) of both familial and sporadic cases of this uniformly fatal disease are characterised at autopsy by TDP-43 proteinopathy in which TDP-43 clears from the nucleus and aggregates in the cytoplasm(4). There are well-established clinical, genetic and pathological overlaps between ALS and frontotemporal dementia (FTD), which together constitute the 'TDP-43 proteinopathies'.

5.2. Drug repurposing based on pre-clinical models of ALS

Currently, there are no drug treatments which significantly affect the natural history and overall prognosis of ALS. Riluzole, in use since 1996, has a modest effect in prolonging survival by a few months, but does not improve symptoms(5, 6). Despite major advances in our understanding of the genetics and cellular pathology of ALS, there has been a succession of failed clinical trials of drugs which have shown evidence of neuroprotection in ALS models. A range of pre-clinical models of ALS (including cells in culture, and on tractable animal models such as zebrafish) have been used to screen drugs which already have regulatory approval for evidence of neuroprotection. However, a major challenge for the field is how to decide which drugs showing promise in pre-clinical models should be taken forward into clinical trials. The poor track record of translating pre-clinical advances to successful clinical trials and effective therapies means there is an urgent need to develop more effective methods to further validate a drug which shows promise from pre-clinical research before proceeding to large scale efficacy trials, to avoid the financial and human cost of negative drug trials. Efficacy studies, which require sample sizes typically of several hundred patients treated over 1-2 years, are hugely expensive and therefore very challenging without commercial backing from major pharmaceutical companies(7). This is a particular issue for drug repurposing studies, where the agent will usually be out of patent.

5.3. Terazosin

An approach that has been shown to confer neuroprotection across a number of different pre-clinical models of neurodegeneration is targeting the glycolytic enzyme PGK1, which improves cellular health by enhancing ATP production. This can be achieved using genetic tools, but importantly can also be brought about by treatment with an FDA/EMA-approved compound, terazosin, which has been shown to confer neuroprotection in animal models of stroke (8) and Parkinson's disease (9).

We have recently found that elevating or activating levels of PGK1 using terazosin ameliorates motor neuron pathology and neuromuscular functional defects in zebrafish models of ALS (based on alterations in ALS-causing genes TDP-43, C9orf72 and FUS), which we have further validated in an ALS cellular model system of primary motor neurons carrying a TDP-43 mutation. Importantly, Cai and colleagues also reported that patients with Parkinson's disease who had been taking terazosin for other clinical indications had slower disease progression and decreased disease-associated complications (9). Thus, we now consider that a pilot study of terazosin in ALS patients is warranted, to determine whether this compound can provide neuroprotection and therefore should be taken forward into clinical trials to determine whether this is matched by therapeutic benefits.

Terazosin is an oral medication given once daily at a typical dose of 2-10mg per day, currently approved for the treatment of hypertension and benign prostatic hypertrophy due to its alpha-adrenergic antagonistic effect. Terazosin has been well-tolerated in previous clinical trials, including in normotensive patients with benign prostatic hypertrophy (10). The most common adverse effect is postural hypotension leading to dizziness (occurring in approximately 14% of subjects compared to 6% on placebo), but occasionally syncope. Other reported adverse effects of terazosin include lethargy (8% terazosin vs 4% placebo) and headache (5% terazosin vs 4% placebo). Each patient will take terazosin for up to 6 months including within-patient dose titration.

5.4. Using biomarkers to inform drug trials

A major barrier to the translation of potential therapies for ALS is the large size and long duration of clinical trials that must be undertaken to identify differences in typical clinical outcome measures based on the accrual of disability and survival. Costs are prohibitively high and evidence of efficacy in early-phase trials often limited. Over the last decade, biochemical measures of the pathological processes occurring in ALS have emerged. We and others have shown that levels of neurofilament and chitinase proteins are tightly linked to disease activity, and show stability in ALS patients over time. Alterations in the levels of these proteins in response to treatment has been demonstrated in other neurological diseases, such as multiple sclerosis, and more recently in ALS trials (11, 12). Changes in the levels of these proteins are detectable over a smaller timescale than alterations in clinical parameters, and due to their stability, the effect of treatment can be assessed by longitudinal measurement in a single group without the need for a control group.

We aim to enrol 50 patients with ALS from the Oxford MND clinic into a clinical study, with key readouts being a group level comparison of neurofilament (13) and chitinase protein levels (14) in plasma and cerebrospinal fluid (CSF) at several timepoints (T0, 3 months and 6 months).

Whilst a demonstrable difference in biomarker levels provides evidence that a drug is favourably influencing the disease process, it is not sufficient to permit immediate translation to clinical use and appropriately powered controlled trials will subsequently be necessary to assess the effectiveness of terazosin on clinical outcome measures. It is anticipated that the results of this study will be used as the basis for the inclusion of terazosin in larger platform therapeutic trials in ALS and encourage the more widespread use of biomarkers as a means to detect efficacy in small early-phase clinical trials of other drugs in future.

6. OBJECTIVES AND OUTCOME MEASURES

	Objectives	Outcome measures	Time points
Primary	To evaluate the futility of terazosin as treatment for ALS using change in plasma neurofilament levels	Proportion of participants with >30% decrease in plasma neurofilament light chain at 6 months, compared with baseline levels	Baseline and 6 months
Secondary	1. To measure change in neurodegeneration, microglial and energy metabolism biomarkers in relation to clinical measures and terazosin treatment in patients with ALS 2. To measure the frequency of adverse events in ALS patients treated with terazosin	1a. Linear models of neurodegeneration and energy metabolism markers: • CSF: neurofilament light chain, phosphorylated neurofilament heavy chain, chitotriosidase 1, chitinase 3-like protein 1, chitinase 3-like protein 2 and PGK1 • Plasma: neurofilament light chain and phosphorylated neurofilament heavy chain • Urine: Titin N-terminal fragment With clinical measures: • Revised ALS Functional Rating Scale (ALSFRS-R) score • Forced Vital Capacity (FVC) • Survival • Cumulative terazosin treatment 1b. Longitudinal changes in biomarker levels comparing baseline with 3 and 6 months 1c. Changes in biomarker and clinical parameter levels in relation to historical control data 2. Adverse events related to IMP and proportion of patients that drop out due to adverse events	Baseline, 3 months and 6 months

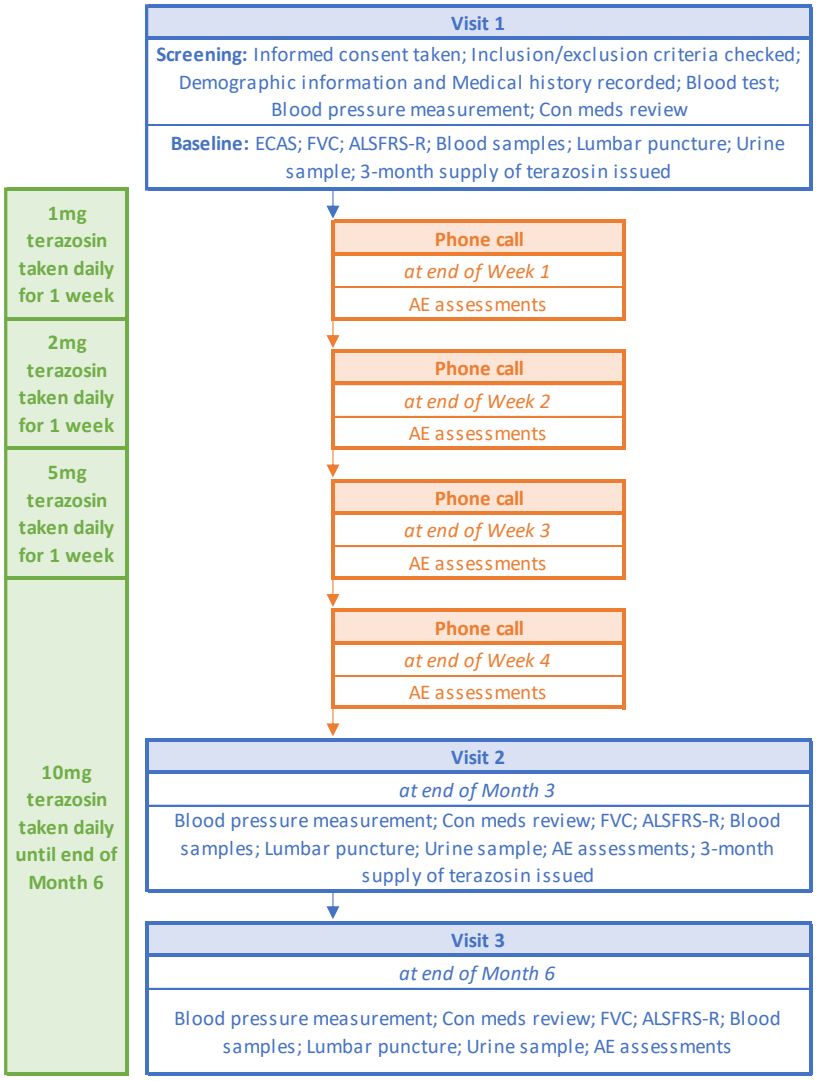
7. TRIAL DESIGN

A single-centre, interventional, open-label, non-randomised, non-controlled proof of concept study to assess the neuroprotective effects of terazosin in patients with ALS.

Participants will be patients with a diagnosis of ALS attending the Oxford MND clinic at the John Radcliffe Hospital (Oxford University Hospitals NHS Foundation Trust). Each participant will take part in the study for up to 6 months, and will receive one tablet of 10mg terazosin per day, titrating up from 1mg per day (as per Section 10.1). Face-to-face study visits will take place at baseline, 3 months and 6 months, coinciding with routine clinical appointments. If the patient's routine appointment is cancelled or delayed by ≥14 days, the study visit should still take place. Patients will undergo venepuncture, lumbar puncture and urine sampling, in addition to routine administration of the ALSFRS-R (Revised ALS Functional Rating Scale) and routine spirometry testing (FVC).

Participants will be asked for permission to access their medical records to record clinical and demographic details for research. Participants will be asked at the time of consent to agree for their samples and anonymised data to be shared with the collaborating institution (University of Edinburgh) for the purposes of this study. If the study samples are not depleted in the analyses or destroyed, they will be stored by the research team. Participants have the option to consent to their anonymised samples being used in future research which has ethics approval (here or abroad, and which may involve commercial organisations).

Chart of the flow of the participant through the study:



8. PARTICIPANT IDENTIFICATION

8.1. Trial participants

Patients with clinically-confirmed ALS attending the Oxford MND clinic.

8.2. Inclusion criteria

1. Participant is willing and able to give informed consent for participation in the study
2. Male or female, aged 18 years or above
3. Diagnosed with ALS (Gold Coast Criteria(15))
4. Symptom onset (first weakness) 9-24 months (inclusive) at enrolment
5. Taking riluzole at a stable dose for at least 4 weeks prior to enrolment, or will refrain from starting riluzole for the duration of the study, or have never taken riluzole
6. Able to swallow tablets safely
7. Willing to use highly effective contraception for the duration of trial treatment and for a duration of 80 days after the last dose

8.3. Exclusion criteria

The participant will not enter the study if ANY of the following apply:

1. Using non-invasive ventilation (NIV)
2. Pregnancy
3. Any other significant disease, disorder, or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data
4. Hypersensitivity to the IMP or any of its excipients (including lactose)
5. Taking terazosin or other alpha adrenergic blockers (doxazosin, prazosin, tamsulosin, silodosin, trazodone, tolazoline, phentolamine, phenoxybenzamine) at time of screening visit or within the 3 months prior to baseline visit
6. Ongoing use of sildenafil, tadalafil, or vardenafil
7. Taking anti-coagulant medication, e.g. warfarin or apixaban
8. Symptomatic postural hypotension or history of postural hypotension
9. Systemic hypotension (systolic BP ≤90mmHg or diastolic BP≤60mmHg)
10. History of micturition syncope
11. Contraindications to lumbar puncture
12. Taking part in a current CTIMP or have taken part in any CTIMP in the 3 months prior to recruitment
13. Known sensitivity to other alpha-adrenoceptor blockers

14. Severe hepatic impairment
15. Cardiac conditions, including: pulmonary oedema due to aortic or mitral valve stenosis, high output cardiac insufficiency, right-sided cardiac insufficiency due to pulmonary embolism or pericardial effusion, left-sided cardiac insufficiency with low filling pressure
16. Participants not willing to follow contraception requirements as detailed in section 11.9
17. Breastfeeding women

9. TRIAL PROCEDURES

Schedule of procedures and interventions:

	Visit 1		Tel call 1	Tel call 2	Tel call 3	Tel call 4	Visit 2	Visit 3
Procedure	**Screening**	**Baseline**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Month 3**	**Month 6**
Informed consent	X							
Inclusion/exclusion criteria	X							
Demographics	X							
Medical history	X							
Blood test (LFT, U&E, FBC, calcium)	X							
Postural blood pressure measurement	X						X	X
Pregnancy test (serum) for WOCBP	X							
Concomitant medications review	X		X	X	X	X	X	X
Physical examination and vital signs	X							
Cognitive assessment (ECAS)		X						
Spirometry test (FVC)		X					X	X
ALSFRS-R		X					X	X
Blood samples for biomarker analysis		X					X	X
Lumbar puncture for biomarker analysis		X					X	X
Urine sample for biomarker analysis		X					X	X
Adverse event assessments			X	X	X	X	X	X
3-month supply of terazosin issued		X					X	

9.1. Recruitment

Potential participants will be identified by routine review of medical notes by the Neurology clinic team, (who all hold substantive or honorary clinical contracts, which include direct patient contact, with the Oxford University Hospitals (OUH) NHS Foundation Trust). Potential participants will be invited to take part

in the study via post or email, with a copy of the Participant Information Sheet (PIS) enclosed/attached. The PIS includes information about who to contact if they have any questions about the study. If they are interested in taking part, a member of the research team will proceed to the screening and eligibility assessment.

9.2. Informed consent

The participant must personally sign and date the latest approved version of the Informed Consent Form (ICF) before any study-specific procedures are performed. Alternatively, if the participant is unable to provide written consent due to physical disability, a witness (other than the person taking consent) will be present at the informed consent discussion and sign the consent form on the participant's behalf.

A small proportion of patients with MND develop cognitive impairments that have overlap with frontotemporal dementia and may impair capacity (<5%). Patients who lack the ability to provide informed consent themselves due to mental capacity are ineligible for the study. Participants who lose capacity to consent during the course of the study will be withdrawn from the study.

Written and verbal versions of the PIS and ICF will be presented to the participants, detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the investigator, their GP or other independent parties to decide whether they will participate in the study. This is in part facilitated by sending the study information ahead of the appointment. Written informed consent will then be obtained by means of dated signatures of the participant and of the person who presented and obtained the informed consent. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Chief Investigator. The original signed ICF will be retained at the study site, a copy will be given to the participant, and another copy will be filed in the participant's medical records.

9.3. Screening and eligibility assessment

The screening assessment (Visit 1) will involve the following:

- Taking informed consent (as described in Section 9.2)
- Checking inclusion/exclusion criteria (each participant must satisfy all the approved inclusion and exclusion criteria of the protocol)
- Recording demographic information and relevant medical history (date of birth, sex, ethnicity, weight, height, family history of neurological disease, medication history, diagnosis, comorbidities, date of symptom onset, date of diagnosis, site of symptom onset)
- Blood test to measure liver and kidney function, full blood count and calcium level
- Measurement of postural blood pressure
- Pregnancy test (serum) for WOCBP (woman of childbearing potential, defined as: fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.)
- Review of concomitant medications
- Physical examination and vital signs

The following additional safety monitoring assessments should be conducted (as a minimum) if clinically indicated: ECG and coagulation tests.

Rescreening will be permitted, using the criteria described above. Rescreening is required if ≥4 months have elapsed since the initial screening.

9.4. Registration/Enrolment

The participant will then be registered/enrolled onto the study using the OCTRU web-based registration system.

There is no randomisation in this study.

As this is a CTIMP, there will be confirmation from a medically qualified clinician for an individual to be entered into the trial.

9.5. Baseline assessments

The baseline assessment (Visit 1) will involve the following:

- Cognitive assessment (Edinburgh cognitive and behavioural ALS screen, ECAS)*
- Spirometry test (FVC)*
- ALSFRS-R score*
- Blood samples (40ml)*
- Lumbar puncture (20ml CSF sample)*
- Urine sample (20ml)*
- 3-month supply of terazosin issued

*as described in Section 9.7

9.6. Subsequent visits

9.6.1 Telephone calls

The patient will be telephoned by a medically-qualified researcher at 1-, 2-, 3- and 4-weeks post-baseline, to review concomitant medications and adverse events, corresponding with the time period during which the terazosin dose is increased from 1mg to 10mg (see Section 10.1). Based on any side effects experienced by the participant, the researcher will advise whether to increase the dose, maintain the current dose or cease taking any trial treatment. If participants experience symptoms indicative of mild postural hypotension on standing (e.g. mild dizziness, mild light-headedness), they will be advised to proceed with dose increase if willing. If, however, participants experience severe dizziness or severe light-headedness (recurrent on standing, or persistent), they will be advised to cease trial treatment or (if taking 10mg) reduce the dose to 5mg.

9.6.2 Visit 2

This visit will take place in clinic at 3 months post-baseline (+/-14 days) and will involve the following:

- Postural blood pressure measurement
- Concomitant medications review
- Spirometry test (FVC)*
- ALSFRS-R score*
- Blood samples (40ml)*
- Lumbar puncture (20ml CSF sample)*
- Urine sample (20ml)*
- Adverse event assessments
- 3-month supply of terazosin issued

The following additional safety monitoring assessments should be conducted (as a minimum) if clinically indicated: physical examination, vital signs, ECG and blood tests (LFT, U&E, FBC, calcium, coagulation tests and pregnancy test).

9.6.3 Visit 3

This visit will take place in clinic at 6 months post-baseline (+/-14 days) and will involve the following:

- Postural blood pressure measurement
- Concomitant medications review
- Spirometry test (FVC)*
- ALSFRS-R score*
- Blood samples (40ml)*
- Lumbar puncture (20ml CSF sample)*
- Urine sample (20ml)*
- Adverse event assessments

The following additional safety monitoring assessments should be conducted (as a minimum) if clinically indicated: physical examination, vital signs, ECG and blood tests (LFT, U&E, FBC, calcium, coagulation tests and pregnancy test).

*as described in Section 9.7

9.7. Description of study procedures

9.7.1 Spirometry test (FVC)

Forced vital capacity is the measurement of the volume of air forcibly exhaled from full inspiration and is a measure of diaphragmatic and intercostal muscle function. Participants will be asked to inhale as far as possible then exhale as hard and as long as possible into a spirometer, which measures the volume of air exhaled. The measurement is adjusted for height and age, and is repeated 3 times with the best response recorded. If the FVC has been administered as part of the patient's coinciding routine clinical appointment, it does not need to be administered during the study visit (the result obtained during the routine clinical appointment will be entered in the CRF).

9.7.2 ALSFRS-R score

The revised ALS functional rating scale (ALSFRS-R) score is a function-based symptom questionnaire comprising 12 questions examining lower limb, upper limb, bulbar and respiratory function. Each question scores between 0 and 4 points with a maximum overall score of 48 (normal function). Scoring will be performed by a delegate trained in administration of the ALSFRS-R. The ALSFRS-R declines in an approximately linear fashion in individuals with ALS. If the ALSFRS-R has been administered as part of the patient's coinciding routine clinical appointment, it does not need to be administered during the study visit (the result obtained during the routine clinical appointment will be entered in the CRF).

9.7.3 Blood test

40ml of blood will be taken by venesection and the plasma separated (the remainder will be destroyed).

9.7.4 Lumbar puncture

20ml will be taken by standard aseptic lumbar puncture procedure in the day investigation unit of the Neurosciences ward at the John Radcliffe Hospital by a trained member of staff. The participant's suitability for the lumbar puncture procedure will be assessed by the investigator before each lumbar puncture timepoint – if any contraindication to the procedure is present the procedure will be not conducted.

Participants who do not wish to undergo lumbar puncture will not be required to do so and will remain eligible to continue in the study.

9.7.5 Urine sample

The patient will be asked to provide a 20ml sample of mid-stream urine at the time of the visit.

9.7.6 Postural blood pressure

In accordance with Royal College of Physicians Guidelines (16), where possible, the patient will be laid supine for at least 5 minutes and the blood pressure measured using an automated sphygmomanometer. The patient will then be asked to stand, or sit if unable to stand, and the blood pressure recorded within 1 minute and again after standing for 3 minutes. Patients unable to lie or stand will have their blood pressure measured only in the seated position. A patient will be classed to have a significant drop in postural blood pressure if they incur one of the below in the measurements taken at 1 or 3 minutes:

- a drop in systolic BP of 20mmHg or more (with or without symptoms)
- a drop in systolic BP to below 90mmHg on standing even if the drop is less than 20mmHg (with or without symptoms)
- a drop in diastolic BP of 10mmHg with symptoms (although clinically less significant than a drop in systolic BP)

9.7.7 ECAS

The Edinburgh cognitive and behavioural ALS screen (ECAS) is a 130-point cognitive screening tool that is adapted for use in people with difficulty communicating or writing due to articulation. It takes 15-20 minutes to complete. The ECAS will be administered face-to-face by a delegate trained in its use. The ECAS is sometimes administered as part of the routine clinical appointment, in which case we would use the results from this routine administration.

9.7.8 Investigational assays

NFL, pNFH, CHIT1, CHI3L1, CHI3L2 and PGK1 will be measured in CSF (pNFH and NFL in plasma) using enzyme-linked immunosorbent assay as per standard protocols. Titin N-terminal fragment:creatinine ratio will be measured in urine. The panel of biomarkers and assays may be expanded during the lifetime of the trial, for example if new evidence arises.

9.8. Sample handling

Blood, CSF and urine samples will be taken at the baseline, 3-month and 6-month visits, as described in Section 9.7. The sample handling procedures will be fully described in the Sample Handling Manual.

Leftover samples will be stored by the study team until the end of the trial (with consent) with a view to performing further assays of interest on these samples.

If the study samples are not depleted in the analyses or destroyed, they will be stored by the research team. Participants have the option to consent to their anonymised samples being used in future research. Any future research will be ethically approved, it may take place here or abroad, and it may involve commercial organisations.

The study samples are **not** “relevant material” under the Human Tissue Act 2004.

9.9. Early discontinuation/withdrawal of participants

During the course of the trial a participant may choose to stop taking the trial treatment or to withdraw from the study:

- Early discontinuation where the participant stops taking treatment but carries on with active follow-up and allows the trial team to continue to access their medical records and any relevant hospital data that is recorded as part of routine standard of care.

- Early discontinuation where the participant stops taking treatment and does not want to participate in active follow-up but allows the trial team to continue to access their medical records and any relevant hospital data that is recorded as part of routine standard of care.
- For withdrawals, the participant withdraws from the study, but data and samples obtained up until the point of withdrawal are retained for use in the study analysis. No further data or samples would be collected after withdrawal.

In addition, the investigator may withdraw a participant from the treatment or study at any time if the investigator considers it necessary for any reason including but not limited to:

- Pregnancy (in which case, the pregnancy will be followed-up to outcome)
- Ineligibility (either arising during the trial or retrospectively, having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or trial requirements
- An adverse event which requires discontinuation of the trial medication or results in inability to continue to comply with trial procedures (in which case, the event will be followed up by telephone until it has resolved)
- Disease progression which requires discontinuation of the trial medication or results in inability to continue to comply with trial procedures

Participants who cannot tolerate the 1mg, 2mg or 5mg dose will be withdrawn from treatment for safety reasons. Participants who cannot tolerate the 10mg dose can remain in the study and on treatment at 5mg.

Participants who commence taking riluzole, or revise their riluzole dosage, during the course of the study, would be withdrawn from the study.

Participants who lose capacity to consent during the course of the study will be withdrawn from the study.

If the lumbar puncture procedure fails for technical reasons, participants can remain in the study (even if this occurs at baseline). A participant's refusal to undergo lumbar puncture at the 3-month or 6-month visit will not constitute a reason for withdrawal.

Ineligible participants, and participants withdrawn due to inability to tolerate the 1mg, 2mg or 5mg dose, will be replaced, if still within the recruitment period of the study.

9.10. Definition of End of Trial

The end of the trial is the point at which all data have been obtained (and all queries resolved) for the final patient's 6-month visit, and all biochemical data analysis is complete including data from assays of interest not yet specified but that may be identified during the lifetime of the trial.

10. TRIAL INTERVENTIONS

10.1. Investigational Medicinal Product (IMP) description

Each participant will receive one oral tablet of 1mg per day for 7 days, followed by 2mg per day for 7 days, followed by 5mg per day for 7 days, followed by 10mg per day until 6 months post-baseline visit. The dose will be increased as tolerated. Participants who cannot tolerate the 1mg, 2mg or 5mg dose will be withdrawn from treatment for safety reasons. If the 10mg dose causes symptoms, the 5mg dose will be reverted to. Patients do not need to be titrated down at the end of treatment.

Terazosin is an alpha-blocker routinely prescribed to patients with hypertension or symptoms from an enlarged prostate. The dosing elevation schedule accords with that recommended in the British National Formulary. Due to the risk of postural hypotension, the participant will be instructed to take the first dose on going to bed in the evening.

Dizziness, light-headedness or fainting may occur when standing up quickly from a lying or sitting position. Patients should be advised of this possibility and instructed to lie down if these symptoms appear and then sit for a few minutes before standing to prevent their recurrence. If syncope occurs the patient should be placed in a recumbent position and supportive treatment applied as necessary. The study investigator should be immediately informed. The participant should be managed as clinically indicated and terazosin treatment must be permanently discontinued as syncope will be considered a severe adverse event.

Terazosin has a marketing authorisation in the UK. It comes in tablet form. Appearance and packaging depend on brand. Terazosin will be sourced by the OUH Clinical Trials Pharmacy.

At the baseline visit, participants will receive clearly labelled: 7 tablets of 1mg terazosin, 7 tablets of 2mg terazosin, 7 tablets of 5mg terazosin, and 3 packets of 28 tablets of 10 mg terazosin. Participants who remain on the 5mg dose will be sent a supply of 5mg tablets via courier (who would collect their surplus 10mg tablets at the same time). At their 3-month visit they will receive a further 4 packets of 28 tablets of 5 mg or 10 mg terazosin. Clinical Trials Pharmacy will perform accountability and reconciliation checks (counting and recording), compliance checks and reconciliation of individual patient supplies (returns). Clinical Trials Pharmacy will add a reduced Annex 13 label at dispensing.

10.1.1. Storage of IMP

Do not store above 25°C, and store in the original package in order to protect from light. The storage conditions recommended by the manufacturer will be followed by pharmacy and instructions on storage given to participants.

10.1.2. Compliance with trial treatment

Participants will be asked to bring all used, unused and part-used packaging to each visit to allow assessment of adherence to the medication schedule. Significant non-compliance will be defined as a participant taking fewer than 80% of doses between study visits (for participants who withdraw from treatment, this will only apply to the period before withdrawal from treatment). To account for non-compliance, secondary analysis will be performed excluding data for participants with significant non-compliance. Returned, expired and unused stock will be destroyed at site.

10.1.3. Accountability of the trial treatment

A risk-adapted approach will be used, using standard prescribing practice. No special accountability required.

10.1.4. Concomitant medication

Caution is advised for the concomitant administration of terazosin with thiazides or other antihypertensive medications, due to the risk of an excessive decrease in blood pressure. Caution is also advised for concomitant administration of terazosin with drugs which may influence hepatic metabolism. Participants who are taking these concomitant medications will be warned that there may be a higher risk of side effects of terazosin.

Concomitant use of phosphodiesterase-5-inhibitors (e.g. sildenafil, tadalafil, vardenafil) with terazosin may lead to symptomatic hypotension in some patients – use of these drugs is therefore an exclusion criterion.

10.1.5. Post-trial treatment

Since this is a proof of principle study, not powered to determine clinical benefit, the drug will not be provided beyond the study period.

10.2. Other treatments (non-IMPS)

Riluzole, if and as prescribed by the patient's treating clinician. Riluzole has a marketing authorisation in the UK and is routinely prescribed to patients with ALS. Note participants do not have to be taking riluzole to participate in the trial.

11. SAFETY REPORTING

Safety reporting for each participant will begin from day of their consent until the participant's final visit.

Events will be followed up until the event resolves or until a cause external to the study is attributed.

11.1. Adverse event definitions

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial 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 trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: ▪ results in death ▪ is life-threatening ▪ requires inpatient hospitalisation or prolongation of existing hospitalisation ▪ results in persistent or significant disability/incapacity ▪ consists of a congenital anomaly or birth defect* Other 'important medical events' may also be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. NOTE: 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. *NOTE: Pregnancy is not, in itself, an SAE. In the event that a participant or his/her partner becomes pregnant whilst taking part in a clinical trial or during a stage where the foetus could have been exposed to the medicinal product (in the case of the active substance or one of its metabolites having a long half-life) the pregnancy should be followed up by the investigator until delivery for congenital abnormality or birth defect, at which point it would fall within the definition of "serious".
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the Reference Safety Information for the medicinal product in question set out: ▪ in the case of a product with a marketing authorisation, in the approved summary of product characteristics (SmPC) for that product ▪ in the case of any other investigational medicinal product, in the approved investigator's brochure (IB) relating to the trial in question

NB: to avoid confusion or misunderstanding of the difference between the terms “serious” and “severe”, the following note of clarification is provided: “Severe” is often used to describe intensity of a specific event, which may be of relatively minor medical significance. “Seriousness” is the regulatory definition supplied above.

11.2. Assessment results outside of normal parameters as AEs and SAEs

A clinically significant drop in postural blood pressure (see definition in Section 9.9.6) is to be reported as an AE.

11.3. Assessment of causality

The relationship of each adverse event to the trial medication will be determined by a medically qualified individual according to the following definitions:

- Definitely related
- Probably related
- Possibly related
- Unlikely to be related
- Not related

For the purposes of reporting, the top 3 definitions (Definitely related, Probably related and Possibly related) will be considered as related to the trial medication.

11.4. Procedures for reporting Adverse Events

All related AEs occurring during the safety window for the trial (as defined above) that are observed by the investigator or reported by the participant will be reported on the trial AE CRF.

The following information will be reported on the CRF: description; date of onset; end date; severity (assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe); assessment of relatedness to trial medication; other suspect drug or device; and action taken. Follow-up information should be provided as necessary.

Non-serious AEs considered related to the trial medication as judged by a medically qualified investigator or the Sponsor will be followed up until the event resolves or until a cause external to the study is attributed.

It will be left to the investigator’s clinical judgment to decide 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 will be followed up by telephone until the AE has resolved, and will continue to be followed up as per routine NHS standard care.

11.5. Procedures for reporting Serious Adverse Events

All SAEs, other than those defined in Section 11.5.1 as not requiring reporting, must be reported on the SAE Form to the Trial Manager immediately or within 24 hours of the Study Team becoming aware of the event being defined as serious.

11.5.1. Events exempt from reporting as SAEs

The following events are not classed as reportable SAEs:

- Hospitalisation for elective procedures planned prior to study entry
- Hospitalisation for standard supportive care for MND, including Percutaneous Endoscopic Gastrostomy (PEG) insertion

- Hospitalisation for lower respiratory tract infection or respiratory failure (unless the investigator considers it exacerbated or related to the IMP administration, in which case it will be reported as an SAE)
- Deaths due to MND (this outcome measure will be captured on the eCRF)

11.6. Expectedness

For SAEs that require reporting, expectedness of SARs will be determined according to the relevant RSI section of the Summary of Product Characteristics (SmPC). The RSI used (within the SmPC) will be the current Sponsor- and MHRA- approved version at the time of the event occurrence. This assessment will be performed centrally by the Nominated Person for the trial.

For assessment of expectedness in the Development Safety Update Report, see Section 11.8 below.

11.7. SUSAR reporting

All SUSARs will be reported by the CTU to the MHRA and to the REC and other parties as applicable. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the CTU is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

Principal Investigators will be informed of all SUSARs for the relevant IMP for all studies with the same Sponsor, whether or not the event occurred in the current trial.

11.8. Development Safety Update Reports

The CI will submit (in addition to the expedited reporting above) DSURs once a year throughout the clinical trial, or on request, to the Regulator (MHRA), Ethics Committee, HRA (where required), Host NHS Trust and Sponsor.

For assessment of expectedness of any SARs in the DSUR, the RSI that was approved **at the start of the safety reporting period** will be used as per OCTRU's relevant SOP. When there has been approved changes to the RSI by substantial amendment during the reporting period, the RSI used for the DSUR will differ to the RSI used to assess expectedness at the time of SAR occurrence for SARs which require expedited reporting.

11.9. Contraception and pregnancy

All participants must be willing to use effective contraception for the duration of trial treatment and for a duration of 80 days after the last dose.

Birth control methods which can achieve a failure rate of less than 1% per year when used consistently and correctly may be considered as highly effective. Such methods include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal)
- progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable)
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomy (provided there has been medical assessment of the surgical success)
- sexual abstinence (sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject)

Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.
Should a study participant become pregnant or aid in the conception of a child (i.e. fathering a child) within 80 days of receiving the IMP, a pregnancy notification form must be completed and submitted as per CRFs. All notified pregnancies will be followed up to birth. Any SAEs deemed to be related to the IMP will be processed as per the SAE reporting process.

12. STATISTICS

12.1. Statistical Analysis Plan (SAP)

The plan for the statistical analysis of the study is outlined below. There is no separate SAP document in use for the study.

12.2. Description of statistical methods

Descriptive statistics of baseline variables will be produced: median, range, interquartile range for variables age at symptom onset, latency from symptom onset to diagnosis, baseline ALSFRS-R score and disease progression rate (calculated as (48-ALSFRS-R score) / months from symptom onset), ECAS score (ALS-specific and total score); proportions for sex, site of onset (upper limb, lower limb, bulbar, other).
The primary analysis will be a single-arm single-stage analysis to consider futility of terazosin treatment based on biomarker response. The primary outcome measure will be plasma neurofilament change at 6 months, with a response defined as a >30% reduction in plasma NFL levels by 6 months compared with baseline levels. In historical control data from two large longitudinal cohort studies, A Multicentre Biomarker Resource Strategy in ALS (AMBRoSIA) [data currently in preparation] and the Clinical Research in ALS and Related Disorders for Therapeutic Development (CReATe) Consortium, the proportion of participants experiencing a 30% fall in plasma NFL at 6 months is 9% (95% confidence interval 5-16%)(17). We therefore consider a response rate of <10% (i.e. less than 10% of participants having a 30% fall in plasma NFL) to be undesirable and a response rate of >25% to be satisfactory, using a significance level of 0.1.
Secondary analysis will examine biomarker responses in CSF NFL and CHIT1, and clinical response in the ALSFRS-R, using a similar dichotomised response based on historical data from AMBRoSIA and, in the case of ALSFRS-R, historical data from the Pooled Resource Open-access Resouce in ALS Clinical Trials (PRO-ACT)(18). Due to the longitudinal stability of the outcome measures, analysis will be performed using longitudinal analysis methodologies. distributions will be examined for normality and, where necessary transformed. Paired analysis (e.g. paired t-test or Wilcoxon signed-rank test) will be used to compare levels at baseline with 3 months and baseline with 6 months.
Exploratory analysis will use longitudinal linear mixed-effects models to measure longitudinal trajectory of all analyte levels and ALSFRS-R to permit controlling for clinical factors that may influence this such as age, gender, symptom onset site and latency from symptom onset to enrolment as well as medication compliance, maximum tolerated and total cumulative terazosin dose in order to explore dose-response effects of terazosin. Where possible, anonymised data will be combined with historical control data from AMBRoSIA (REC ref: 16/LO/2136), CreATe and PRO-ACT for these analyses.
Descriptive statistics will be produced regarding adverse events.

12.3. Sample size determination

Based on the primary futility analysis approach, 31 patients will give 80% power to detect futility at 10% significance, given a minimum acceptable 25% response rate, using an exact binomial test.

We have selected a sample size of 50 to allow for participants who intolerant of treatment, withdraw from the study or decline follow up lumbar puncture, and to allow for increased variability of the other analytes included in the analysis.

12.4. Analysis populations

Analysis of the primary and secondary outcomes will be performed on all participants who started treatment and for whom follow up data are available. Additional analysis will be performed excluding participants with significant non-compliance (defined as <80% compliance) or who do not reach the maximum dose of terazosin. As outlined above, compliance and total terazosin exposure will be included as variables in linear mixed effects models.
Analysis of adverse events will be performed using all participants who started treatment.

12.5. Decision points

No interim analysis will be performed.

12.6. Stopping rules

There are no planned stopping rules.

12.7. The level of statistical significance

5% two sided. 95% confidence intervals will be reported.

12.8. Procedure for accounting for missing, unused and spurious data

All available data will be used for the primary analysis and for longitudinal analysis. Missing data will be minimised as far as possible. Sensitivity analysis will be performed using different imputation techniques will be performed to account for missing-not-at-random data, including last observation carried forward and multiple imputation by chained equations.

12.9. Procedures for reporting any deviation(s) from the original statistical plan

Any deviation(s) from the original statistical plan outlined will be described and justified in the protocol or the final report, depending on the timing of the changes.

13. DATA MANAGEMENT

The data management aspects of the study are summarised here with details fully described in the Data Management Plan.

13.1. Source data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions.

On all trial-specific documents, other than the signed consent, the participant will be referred to by the trial participant number/code, not by name.

13.2. Access to data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

13.3. Data recording and record keeping

All trial data will be entered into a validated REDCap database developed and maintained by OCTRU and which can only be accessed by authorised users via the application. The application resides on a webserver hosted and managed by the University of Oxford's Medical Services Division IT Services department (http://www.imsu.ox.ac.uk/). The server is on the university's backbone network and is backed up nightly to a secure off-site location.

The participants will be identified by a unique trial-specific number and/or code in any database. The name and any other identifying detail will NOT be included in any study data electronic file.

Research data will be stored for 20 years after the end of the study.

The paper consent forms will be retained for the life of the samples to meet HTA traceability requirements.

Participants will be given the option to be approached for future research, and as such the paper consent forms will be retained as the basis for retention of details and future approach. Those contact details will be held securely, separately from the research data, and kept updated.

13.4. Data sharing

Data collected during the course of the study may be passed on to academic and/or commercial partners, in anonymised form. Anonymised data may also be shared abroad, with countries with different data protection regulations.

14. QUALITY ASSURANCE PROCEDURES

14.1. Risk assessment

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and Standard Operating Procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities.

14.2. Monitoring

Regular central monitoring will be performed according to the trial-specific Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents as these are defined in the trial-specific Monitoring Plan. Following written Standard Operating Procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

14.3. Trial committees

As this is a small proof-of-concept trial, a formal TSC and DSMC will not be formed. There will however be a Trial Oversight Group (TOG) formed.

14.3.1 Trial Management Group

The Trial Management Group (TMG) will meet on a monthly basis throughout the trial to discuss the practicalities of running the trial and to monitor the conduct and progress of the trial. The TMG consists of the individuals responsible for the operational management of the trial, including the CI, Trial Manager and key members of the scientific and clinical teams.

14.3.2 Trial Oversight Group

The TOG will comprise two independent clinicians experienced in the field of MND clinical trials, along with an independent statistician. The TOG will work closely with the TMG and will provide overall supervision of the trial through its independent chair.
The TOG's responsibilities will include:

- To provide overall supervision of the trial and ensure it is being conducted in accordance with GCP
- To advise on protocol development and ensure adherence to the protocol during the trial period
- Establish frequency of meetings prior to commencement of trial
- To provide advice to the CI, TMG and (if appropriate) funder
- To review AEs and SAEs reported by the CI
- To monitor safety of study participants and suggest any amendments to the protocol or termination of the trial if deemed necessary for patient safety

15. PROTOCOL DEVIATIONS

A trial related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g. consent process or IMP administration) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form, filed in the trial master file and reported in the final study report.

16. SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial."

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory authority and the relevant NHS host organisation within 7 calendar days.

17. ETHICAL AND REGULATORY CONSIDERATIONS

17.1. Declaration of Helsinki

The investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

17.2. Guidelines for Good Clinical Practice

The investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

17.3. Approvals

Following Sponsor approval the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), HRA (where required), regulatory authorities (MHRA in the UK), and host NHS institution for written approval.

The investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

17.4. Other ethical considerations

The study involves clinical assessments, lumbar puncture and blood-sampling, over a period of 6 months (3 visits), and it is recognised that all parts, specifically lumbar puncture, may not be practical due to disability. It will be made clear therefore that it is not necessary for participants to undergo lumbar puncture at each timepoint.

CSF extraction by lumbar puncture will only be carried out by a team member with experience and training to do so, and with strictly aseptic technique. Previous experience suggests that the local rates for any adverse event (e.g. mild pain or post-procedure headache) are extremely low (<5%, which is significantly lower than the national average). An LP requires the injection of a small amount of subcutaneous local anaesthetic at the base of the spine, which is frequently mildly painful for several seconds before anaesthesia is achieved. It is not always possible to achieve full anaesthesia, and occasionally the patient will experience a transient tingling sensation in the lower limb during entry to the dural sac. Removal of even the small volume of CSF proposed (up to 20ml, which is <10% of the total volume at any one time, and <10% of the daily volume produced) can result in a post−LP headache of varying severity. This is typically mild and self-limiting after 24-72 hours. It tends to affect patients <30 years of age who would not be the typical demographic of the study. We aim to use atraumatic needles (e.g. Whitacre, Sprotte), or alternatively no larger than 22G Quincke-tip, to minimise this risk. In very rare cases (<1/year in the routine clinical setting of a busy neurological ward)(19) the headache can be severe enough to require hospital admission and a further invasive procedure in the form of a 'blood patch'. This would be performed by a trained anaesthetist. In exceptionally rare circumstances in the global literature (and not expected within our proposed study), an LP can result in the potentially life−threatening conditions of subdural haematoma (requiring craniotomy to relieve intracranial pressure), or meningitis (requiring intravenous antibiotics). All necessary expertise is available on the same site to deal promptly with such remote outcomes. Blood-taking (up to 40ml) involves a minor transient pain from the needle and occasionally localised bruising. Only trained individuals and sterile technique will be used.

It is highly unlikely that any incidental findings of clinical significance will be made in the analysis. Should this occur, however, the incidental findings will be fed back to the participant's GP.

17.5. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, MHRA, HRA (where required), host organisation, funder (where required) and Sponsor. In addition, an End of Trial notification and final report will be submitted to the MHRA, the REC, host organisation and Sponsor.

17.6. Transparency in research

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database.

Results will be uploaded to the European Clinical Trial (EudraCT) Database within 12 months of the end of trial declaration by the CI or their delegate.

Where the trial has been registered on multiple public platforms, the trial information will be kept up to date during the trial, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the trial declaration.

17.7. Participant confidentiality

The study will comply with the UK General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be anonymised as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all study documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

17.8. Expenses and benefits

Participants will be reimbursed reasonable travel expenses where necessary.

18. FINANCE AND INSURANCE

18.1. Funding

The study is funded by the My Name'5 Doddie Foundation (registered charity number: SC 047871). The grant that funds this study is also funding analysis of biomarkers of ALS in mouse models by our collaborators at the University of Edinburgh, to which the results from this study will be compared.

18.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

18.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

19. PUBLICATION POLICY

The investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the My Name'5 Doddie Foundation. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

20. ARCHIVING

The Trial Master File and trial data will be archived within the University of Oxford on secure servers for a period of 20 years from the end of the study.

21. REFERENCES

1. Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, et al. Amyotrophic lateral sclerosis. Nature reviews Disease primers. 2017;3(1):1-19.
2. Zou Z-Y, Zhou Z-R, Che C-H, Liu C-Y, He R-L, Huang H-P. Genetic epidemiology of amyotrophic lateral sclerosis: a systematic review and meta-analysis. Journal of Neurology, Neurosurgery & Psychiatry. 2017;88(7):540-9.
3. J.P. T, R.H. B. Decoding ALS: from genes to mechanism. Nature. 2016;539:197-206.
4. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science. 2006;314(5796):130-3.
5. Fang T, Al Khleifat A, Meurgey J-H, Jones A, Leigh PN, Bensimon G, et al. Stage at which riluzole treatment prolongs survival in patients with amyotrophic lateral sclerosis: a retrospective analysis of data from a dose-ranging study. The Lancet Neurology. 2018;17(5):416-22.
6. Lacomblez L, Bensimon G, Meininger V, Leigh P, Guillet P. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. The Lancet. 1996;347(9013):1425-31.
7. Talbot K, Feneberg E, Scaber J, Thompson AG, Turner MR. Amyotrophic lateral sclerosis: the complex path to precision medicine. Journal of neurology. 2018;265(10):2454-62.
8. Chen X, Zhao C, Li X, Wang T, Li Y, Cao C, et al. Terazosin activates Pgk1 and Hsp90 to promote stress resistance. Nat Chem Biol. 2015;11(1):19-25.
9. Cai R, Zhang Y, Simmering JE, Schultz JL, Li Y, Fernandez-Carasa I, et al. Enhancing glycolysis attenuates Parkinson's disease progression in models and clinical databases. J Clin Invest. 2019;129(10):4539-49.
10. Yuan J, Liu Y, Yang Z, Qin X, Yang K, Mao C. The efficacy and safety of alpha-1 blockers for benign prostatic hyperplasia: an overview of 15 systematic reviews. Curr Med Res Opin. 2013;29(3):279-87.
11. Lu CH, Macdonald-Wallis C, Gray E, Pearce N, Petzold A, Norgren N, et al. Neurofilament light chain: A prognostic biomarker in amyotrophic lateral sclerosis. Neurology. 2015;84(22):2247-57.
12. Thompson AG, Gray E, Bampton A, Raciborska D, Talbot K, Turner MR. CSF chitinase proteins in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2019;90(11):1215-20.
13. Menke RA, Gray E, Lu CH, Kuhle J, Talbot K, Malaspina A, et al. CSF neurofilament light chain reflects corticospinal tract degeneration in ALS. Ann Clin Transl Neurol. 2015;2(7):748-55.
14. Thompson AG, Gray E, Thézénas ML, Charles PD, Evetts S, Hu MT, et al. Cerebrospinal fluid macrophage biomarkers in amyotrophic lateral sclerosis. Ann Neurol. 2018;83(2):258-68.
15. Shefner JM, Al-Chalabi A, Baker MR, Cui LY, de Carvalho M, Eisen A, et al. A proposal for new diagnostic criteria for ALS. Clin Neurophysiol. 2020;131(8):1975-8.
16. Measurement of lying and standing blood pressure: A brief guide for clinical staff 2017 [Available from: https://www.rcplondon.ac.uk/projects/outputs/measurement-lying-and-standing-blood-pressure-brief-guide-clinical-staff.
17. Benatar M, Zhang L, Wang L, Granit V, Statland J, Barohn R, et al. Validation of serum neurofilaments as prognostic and potential pharmacodynamic biomarkers for ALS. Neurology. 2020;95(1):e59-e69.
18. Atassi N, Berry J, Shui A, Zach N, Sherman A, Sinani E, et al. The PRO-ACT database: design, initial analyses, and predictive features. Neurology. 2014;83(19):1719-25.
19. Nath S, Koziarz A, Badhiwala JH, Alhazzani W, Jaeschke R, Sharma S, et al. Atraumatic versus conventional lumbar puncture needles: a systematic review and meta-analysis. The Lancet. 2018;391(10126):1197-204.

22. APPENDIX A: AMENDMENT HISTORY

Amendment no.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made